Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands Sw kr. 170, incl. postage.
Other countries: Sw kr 200 incl. postage.

Chief Editor

Professor Jan Waldenström, MD
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Riddargatan 12 B
S-114 35 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksell Informationsindustri AB
S-751 81 Uppsala, Sweden

CLINICAL AND METABOLIC ASPECTS OF FRUCTOSE

CLINICAL AND METABOLIC ASPECTS OF FRUCTOSE

Papers Presented at a Symposium
in Helsinki, January 20—22 1972

Edited by

ESKO A. NIKKILÄ
JUSSI K. HUTTUNEN

ACTA MEDICA SCANDINAVICA SUPPLEMENTUM 542

Printed in Finland
Vammala 1972, Vammalan Kirjapaino Oy

CONTENTS

FOREWORD

Acta med scand. Suppl. 542, pp. 9—10

FRUCTOSE — SWEET WITHOUT RISK FOR HEALTH?

OPENING OF THE SYMPOSIUM

Esko A. Nikkilä

Fructose is a natural compound which the mammalian body can both synthesize and metabolize. In an adult organism the former process evidently occurs to a very small extent whereas the machinery intended for the assimilation of exogenous fructose has such a high capacity that even total basic energy consumption can be covered by this single dietary constituent without difficulty. In fact, fructose is utilized by the body much more readily and in a less complicated way than glucose. As the nature seldom equips itself with devices which it does not need one should be ready to believe that fructose is intended to form one major natural nutrient of herbivorous and omnivorous animals including man. This view also implies that fructose — in moderate amounts at least — does not have any adverse effects in the body.

In the form of refined sugar fructose has become a favored nutrient the consumption of which has been steadily increasing during this century. As this progress coincides with spread of many diseases, notably most conspicuously with the increase in the prevalence of atherosclerosis and coronary heart disease, it is self-evident that sucrose has been placed on the black list of those exogenous factors which may be related to the genesis of these disorders. Even though there is so far little evidence on a causal association of sucrose consumption and atherosclerosis there are in fact several possible ways by which sucrose could promote atherogenesis. It causes prompt increases of blood glucose and plasma insulin, a combination which is thought to stimulate lipid synthesis in arterial wall, adipose cell and liver resulting in atheroma, obesity and hyperlipemia, respectively. Sugar is intended to be used as a sweetener but actually has become a significant source of calories, which often form just the extra calories leading to excessive increase of adipose tissue. Whether a sucrose-rich diet is also involved in the etiology of diabetes remains unanswered but most data do not support this hypothesis. Of course, sucrose may manifest a latent diabetes more readily than other dietary carbohydrates.

The basic differences in the physiology and metabolic influences of fructose and sucrose were recognized already a century ago when Külz (1874) showed that diabetics can tolerate

fructose much better than sucrose. This early observation was subsequently well confirmed in clinical and experimental studies and it received an adequate explanation when it became clear that fructose neither stimulates insulin release nor needs insulin for its own metabolism in the body. As fructose thus causes no increase of plasma insulin and elevates blood glucose much less than an equivalent amount of sucrose it appears to be devoid of many of the adverse effects of the latter. As fructose furthermore has a good antiketogenic activity it seems to be a useful sweetener for diabetics.

This view is supported also by some controlled clinical trials. However the general opinion of clinicians through years has been against the use of fructose and it has been repeatedly concluded that fructose should not be recommended for diabetics. There is no question that this view is correct for an overweight diabetic to whom no sugar is better than any sugar.

Until recently the use of fructose as an ordinary dietary constituent has been much [illegible]ited by its high price as compared to suc[illegible] A few years ago the chemists at The [illegible] Sugar Company Ltd. succeeded in de[illegible]g a new manufacturing process for fructose and this has made it possible to prepare fructose in industrial scale at a reasonable price. This of course has been followed by marketing and advertising of fructose as a natural sweetener the use of which is more healthy than that of sucrose. The increasing availability and use of fructose, then, gives a challenge to both basic and clinical research for further exploration of the metabolic effects, beneficial or harmful, of dietary fructose in a normal man and in different disorders like diabetes, obesity and hyperlipemia. An additional and highly interesting area of research is formed by the interaction of fructose with the metabolism and metabolic effects of ethanol and by the potential therapeutic use of fructose in ethanol induced disorders like ethanol intoxication, hangover and fatty liver. One further question concerns the influence of fructose on caries.

In parallel with the industrial production of fructose some clinical studies on the use of fructose in the diet of diabetics and of patients with hypertriglyceridemia were initiated in Helsinki. It then appeared that in spite of a rather extensive research on the biochemistry and physiology of fructose no recent review article had been published or any conference held on the subject. This gave the idea for the present symposium of Clinical and Metabolic Aspects of Fructose. The Finnish Sugar Company Ltd. was generous enough in sponsoring the symposium in spite of being well aware of the possibility that most data to be presented were not favorable for the sale of this product. The organizers of the symposium were happy to receive positive responses from almost all of the invited speakers and other participants. In opening the symposium I will direct our appreciation to the sponsor and to the lecturers, who also have prepared their manuscripts in good time to allow a rapid publication of this book.

METABOLISM OF FRUCTOSE AND HEREDITARY DISORDERS OF FRUCTOSE METABOLISM

Acta med. scand Suppl. 542, pp 13—18

INTESTINAL ABSORPTION OF SUCROSE

Arne Dahlqvist

From th Research Department 1 E blocket University Hospital, and th Department f Nutrition Chemical Center Lund, Sweden

Abstract Sucrose is synthetized in the green leaves of plants. With increasing economical status the sucrose from sugar cane and beets, like fat, supplies an increasing fraction of our food. Sucrose is easily metabolized and utilized. Too high consumption is, however not desirable from nutritional point of view since this highly refined product contains calories but no essential nutrients.

The general concept is that animals (and humans) do not synthetize sucrose. A single report of a sucrose-synthetizing patient needs confirmation from other sources before it can be accepted.

The intestinal absorption of sucrose can only occur if the hydrolyzing enzyme, invertase (sucrase), is present in the mucosal cells. Acid hydrolysis in the stomach has been suggested, but does not occur The intestinal invertase is an α-glucosidase.

In the human intestine invertase, as well as the other α-glucosidases, is developed very early in fetal life — much earlier than lactase. In the animals studied so far in contrast, intestinal invertase and other α-glucosidases are weak until the weaning period, when lactase disappears and the α-glucosidase develops. The reason for these species differences is yet unexplained.

Human populations with low sucrose consumption have approximately equally high intestinal invertase activity as those in countries with high sucrose consumption. In Greenland Eskimos the adults have for very long period of time (probably thousands of years) consumed nearly no carbohydrates at all. The average intestinal activity of invertase and the other α-glucosidases in the Greenland Eskimos is nevertheless nearly the same as ours. Specific enzyme defects, which among us occur only as very rare cases of inborn errors of metabolism are, however rather frequent in the Greenland Eskimos.

From chemical point of view sucrose is β-D-fructofuranosyl-α-D-glucopyranoside (Fig. 1) i.e. it is composed of one molecule of fructose and one molecule of glucose, and each one of the two monosaccharides is joined to the other one through a glycosidic link. Sucrose for this reason has a number of chemical pecularities. It is non-reducing, and it is in fact stable against boiling also with very strong solutions of alkali. Due to the fructofuranoside part it is, however much more easily hydrolyzed by acids than is any of the other common disaccharides.

Sucrose is synthesized in the green leaves of probably all plants. It is then stored in the fruits and roots as a source of energy and carbon.

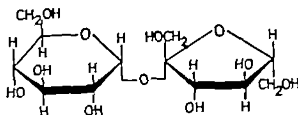


Fig. 1 The chemical structure of sucrose is β-D-fructofuranosyl-α-D-glucopyranoside.

It is not assumed that sucrose can be synthesized in the human body or in animals. However a research group in Moscow (16) some years ago described a patient who was claimed to excrete 100—200 grams of sucrose per day in the urine. This finding was said to confirm reports of a few patients published by German authors in the 1930-ies. No sucrose was found in the blood, and the synthesis of the disaccharide was therefore assumed to occur in the kidneys. The possibility that the patient might deliberately add crose to the urine was considered, but still he patient was kept under careful n o pre ent such addition, the urine continued o ontain sucrose.

Although this report originates from a research group with good reputation, it seems difficult to accept until similar cases have been described by other groups within a reasonable period of time To me it appears quite too likely that a patient, who for some reason wants to simulate diabetes, will try to add sugar to the urine. If she does not have elementary knowledge of biochemistry she may believe that the sugar found in the urine of diabetic patients is the same sugar as we use in tea of coffee, and add that kind of sugar It is also quite likely that the patient may be skilful enough to add sugar to the urine in spite of the doctor's attempts to control this. Urine obtained by catheterization should be analyzed, which does not seem to have been done.

Even if some humans were able to synthesize sucrose, they would hardly be able to compete with our two most important commercial sources of sucrose, namely the sugar cane and the sugar beet, which account for nearly 60 and 40 %, respectively of the total amount of sugar consumed (22).

The amount of sucrose consumed per individual and time, like the consumption of fat, increases when the average income in the country increases. From the official figures it can be calculated that at present the poorest countries, like India, Pakistan and China, consume around 15 g of sucrose a day per individual, and the wealthiest countries, as USA, England, Scandinavia and others, 150 g (22). In the countries with high sucrose consumption sucrose would according to these figures account for nearly one-fourth of the total calorie consumption. This figure is, however probably somewhat overestimated.

From nutritional point of view too high sucrose consumption is not desirable, since it is a highly refined product containing no essential nutrients High sucrose consumption increases dental caries, and according to the concept of some authors it may also increase the incidence of atherosclerosis. Nevertheless both humans and animals have a strong preference for the sweet taste of sucrose and sucrose-containing foods (22)

Like other disaccharides sucrose must be hydrolyzed into its monosaccharide components (i.e. glucose and fructose) before it can be absorbed in the small intestine. There is no significant intestinal transport of unhydrolyzed sucrose, and in animal experiments we have found that sucrose administered by injection is quantitatively excreted in the urine.

Since sucrose is so sensitive to acid hydrolysis, it has been suggested that the hydrolysis of ingested sucrose might start already in the stomach, being catalyzed by the hydrochloric acid We have not found any

Table I. *Disaccharidases and glycosidases in the small intestine. The brush border enzymes account for the digestion of dietary disaccharides while the soluble and lysosomal enzymes probably have other functions.*

A) *Brush border enzymes*
  1) *Isomaltase* This enzyme also hydrolyzes maltose, and accounts for bout 50 / of the total maltase activity
  2) *Invertase (= sucrase)* Also this enzyme hydrolyzes maltose, and accounts for about 25 / of the total maltase activity
  1) *Heat stable maltases*
  4) *Lactase.*
  5) *Trehalase.*

B) *Sol ble or lysosomal enzymes*
  1) *Acid β-galactosidase.*
  2) *Hetero-β-galactosidase*
  3) *Acid α-glucosidase*

evidence for acid hydrolysis of sucrose in the stomach, however when we studied the digestion and absorption of test meals in humans (6). The hydrolysis of sucrose in the digestive tract seems to be catalyzed by enzymes, as is the hydrolysis of other food components. This concept is also supported by the fact that patients with congenital lack of intestinal invertase (sucrase), are unable to digest and absorb sucrose (2, 15, 21).

The small intestine contains a number of disaccharidases (Table I). These enzymes are localized in the brush borders of the mucosal epithelial cells, and exert their function in this location. They are thus *not* secreted. The invertase also hydrolyzes maltose and some other α-glucoside substrates. This and other facts show that the intestinal invertase is an α-glucosidase, in contrast to the invertase in yeast which is a β-fructosidase

The ability of a subject to absorb a specific disaccharide is dependent on the intestinal activity of the enzyme which hydrolyzes the disaccharide in question. It would therefore be of interest to know which factors regulate the activity of the different disaccharidases in the intestine. One apparent possibility is that the presence of disaccharides in the food may stimulate the corresponding disaccharidase activity by adaptation. Some such effects have been observed, but it must be stated that in these cases the effect is relatively weak, and in many cases no effect at all of dietary disaccharides on the intestinal disaccharidase activity could be seen. Therefore *substrate adaptation in the sense usually meant by this expression is not the most important factor in the regulation of the small intestinal disaccharidase activity* Although in some instances changes in the activity of the intestinal disaccharidases seem to coincide with changes in the composition of the diet, the changes in enzyme activity are not produced by these changes of the diet. There are some rather curious species differences in the development of the small-intestinal disaccharidases, which cannot be explained at present, but which may with time throw some light on the regulating factors. So the intestine of a newborn pig has high lactase activity but low activity of invertase and the other α-glucosidase activities (3 4, 7 14). This means if a newborn pig is fed sucrose instead of lactose, it will get diarrhea, and eventually die. During and after weaning the lactase will decrease, and the α-glucosidases increase (Fig 2). These changes closely follow the dietary changes. If milk feeding is continued, however the same changes in enzyme activity will nevertheless occur and therefore the enzyme activity is not directly controlled by the diet, but by some other mechanism, for instance hormonal (12)

In the human the development of the disaccharidases is quite different. The α-glucosidases (including the invertase) develop already during early fetal life (9), and are thus fully developed a long time before birth. The lactase, in contrast, develops rather late in the fetal life (Fig. 3). In North Europeans and North-Americans the high lactase activity present at birth persists throughout life, but in most other human populations the lactase activity decreases markedly after the first years of life, and in those populations the adult human (like the adult animals of the

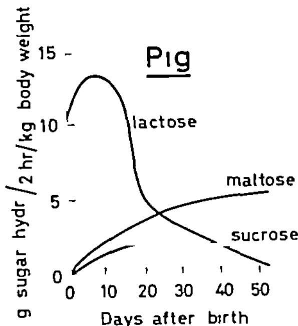


Fig 2 Development of small intestinal disaccharidases in the pig (3, 14)

mammalian species so far studied) is intolerant of lactose (for review see 10—11) Also in these populations, however the α-glucosidases seem t develop far before birth.

If we take a n example the development of lactase in human, the activity of this enzyme in th lult subject is not dependent on the dietary h b ts either of the single subject or of the last few generations of his ancestors. If th dietary habits over a larger period of time are tak n nto consideration, however there is a clearcut correlation between the persistance of intestinal lactase in the adult human and the dietary habits of the population to which he belongs (18, 19) This seems to indicate the results of a natural selection in the sense described by Darwin rather than the result of enzyme adaptation.

Rosenzweig et al. (17) have studied the influence of dietary sugars on the small-intestinal enzyme activity by enzyme activity assays in mucosal biopsies. They found no effect of lactose, but sucrose administration increased to some extent the activity of invertase. Administration of fructose had the same effect. I have earlier stated that there is at least a 10-fold variation between different populations in the average sucrose consumption. It would then be of interest to know whether these differences in sucrose consumption are reflected in the mucosal invertase activity In Table II the mean values for the maltase, invertase, trehalase and lactase activities in one Indian and one Thai group (low sucrose consumption) are compared with the values for two American and one Swedish group (high sucrose consumption) (5 13, 20) The maltase, invertase and trehalase activities are approximately the same in all these groups. The lactase activity in contrast, is much lower in the Indian and Thai groups than in the American and Swedish groups, which is in accord with our previous knowledge (see Table I). The total carbohydrate consumption is comparable in all these populations, since the low sucrose intake in the Indian and Thai groups is compensated with a higher consumption of starch.

We have also studied the intestinal disaccharidase activities in a group of subjects

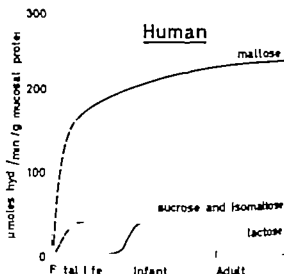


Fig 3 Development of small-intestinal disaccharidases in the human (9)

Table II. *Mean values for disaccharidase activities in populations of high and low sucrose consumption.*

Mean values for disaccharidase activities in studies of normal subjects in India (20) and Thailand (13) which represent populations with low sucrose consumption, compared with American controls (subjects newly arrived to India and Thailand, respectively) and a reference group studied in a Swedish investigation (6). The American and Swedish groups represent populations with very high sucrose consumption. The invertase activity appears to be approximately the same in the high-sucrose and the low-sucrose populations. The lactase activity in contrast, is low in the Indian and Thai groups, which is in accord with earlier experiences.

	Units/gram protein (mean)				
	American controls	Indian	American controls	Thai	Swedes
	n=30	n=20	n=23	n=74	n=37
Maltase	263	224	375	292	258
Invertase (= sucrase)	88	81	108	79	69
Trehalase	43	19	—	—	24
Lactase	26	3.4	38	2.1	29

of a population in which the adults for probably several thousands of years have consumed essentially no carbohydrates at all, namely Greenland Eskimos (1). The findings in these are rather astonishing. The mean values of the different disaccharidases are not very much different from those of the other populations studied (maltase 187 units/gram protein, invertase 50 trehalase 14, and lactase 4.8). Out of 19 adult Eskimos studied, 17 were intolerant for lactose, which only shows that, as should be expected, the Greenland Eskimos belong to those populations in which lactase deficiency is the normal state in the majority of the adults. A surprising fact was, however the frequent occurrence of other specific enzyme deficiencies. In the same group of 19 Eskimos, 3 subjects completely lacked invertase and isomaltase, and 2 subjects showed a nearly complete lack of trehalase. In other populations such specific enzyme deficiencies occur only as very rare congenital defects (inborn errors of metabolism) with a frequency which is probably below 1 10 000. Omission of carbohydrates during a very long period of time from the diet of a population thus does not seem to result in a generally low level of disaccharidase activity in the small intestine, but rather in an increased frequency of specific complete or nearly-complete enzyme defects. It is understandable that in populations in which the adults do not consume carbohydrates such deficiencies do not involve any disadvantages. It is more difficult to find, however any reasons making them more fit to survive.

I have gone into much detail about the regulation of the intestinal disaccharidase activity especially the invertase, and I will therefore have to omit details about some other aspects on the intestinal absorption of sucrose which, however also are very fascinating. I will just mention that there is for instance some yet not fully understood relation between the invertase and isomaltase activities. Although these activities are exerted by different enzyme molecules, these two enzymes seem to be built in together in a common substructure of the microvillous membrane. They also seem to be controlled by a common gene, so if one of the enzymes is absent as the result of a genetic change, the other enzyme is absent too (2). There also seems to be a spatial relationship between the sucrase and a specific membrane carrier for glucose released from sucrose during hydrolysis, giving such glucose a kinetic advantage over free glucose. The carrier is thus different from the carrier for free glucose (6). These data indicate a very delicate organization on the membrane level, which is yet only partly understood. The

absorption of the fructose component of sucrose does not occur via the same carrier as glucose, but recent data indicate an active transport mechanism also for fructose.

REFERENCES

1. Asp, N G Berg, N O Dahlqvist, A., Gudmand Høyer E. & Jarnum, S Intestinal disaccharidases in Greenland Eskimos (Manuscript, 1972)
2. Auricchio S Dahlqvist, A., Mürset, G & Prader A. Isomaltose intolerance causing decreased ability to utilize dietary starch. J Pediat. 62 165, 1963.
3. Bailey C. B., Kitts, W D & Wood, D J.. The development of the digestive system of the pig during its pre-weaning phase of growth. B. Intestinal lactase, sucrase and maltase. Canad. J agric. Sci. 36 51, 1956.
4. Becker D E., Ullrey D E., Terrill, S. W & Notzold, R. A. Failure of the newborn pig to utilize dietary sucrose. Science 120 345, 1954.
5. Berg, N O Dahlq st, A., Lindberg, T & Nordén, Å.. Intestina dipeptidases and di saccharidases in c ia disease in adults. Gastroenterology 5 3 5, 19 0
6. Crane R. K M lathi, P Caspary W F & Ramasw m F A new transport system as the b   f t e kinetic advantage con tributed   sorpti by brush border digesti   4th World Congress of Gast oen C penhagen 1970
Dahlq r l stinal carbohydrases of a ewborn ure (Lond) 190 31, 1961.
8. Dahl st g trom, B. Digestion and bsorpt charides in man. Biochem. J 81 411
9. Dahlq st A Lindberg. T Development of the intest   d accharidase and alkaline phosphatase t ties in the human fetus. Clin Sci 30 51 958
10. Dunphy J V Littman A & Hammond, J B. Intestinal lact d fi ency in adults Gastroentero ogy 49 1 1 65
11. Gudmand Høyer E. Specific Lactose Malabsorption in Adults (Dissertation). Fadl s Forlag, Copenhagen 1971.
12. Hahn, P & Koldovský O Utilization of Nutrients During Postnatal Development. Pergamon Press, Oxford 1966.
13. Keush, G T Troncale, F J Thavaramara, B., Prinyanont, P Anderson, P R. & Bhamarapravathi, N Lactase deficiency in Thailand Effect of prolonged lactose feeding Amer J clin. Nutr 22 638, 1969
14. Kitts, W D Bailey C. B. & Wood, A. J The development of the digestive enzyme system f the pig during its pre-weaning phase of growth. A. Pancreatic amylase and lipase. Canad. J agric. Sci. 36 45, 1956.
15. Prader A., Auricchio, S & Mürset, G. Durchfall infolge hereditären Mangels an intestinaler Saccharaseaktivität (Saccharoseintoleranz) Schweiz. med. Wschr 91 465, 1961.
16. Rosenf ld, E. L., Lukomskaya, I. S., Gorodez ky W K., Zarubina, N A. & Zarezky M. M.. A case of sucrose synthesis in the human organism. Clin. chim. Acta 11 185 1965
17. Rosenzweig, N S., Herman, R. H., & Stifel, F B.. Dietary regulation of small intestinal enzyme ctivity in man. Amer J clin. Nutr 24 65 1971
18. Simoons, F J Primary adult lactose intolerance and the milking habit A problem in biological and cultural interrelations. I. Review of the medical research. Amer J. dig. Dis. 14 819 1969
19. Simoons, F J Primary adult lactose intolerance and the milking habit A problem in biologic and cultural interrelations. II. A culture historical hypothesis. Amer J dig. Dis. 15 695 1970
20. Swaminathan, N Mathan, N Baker S. J & Radhakrishnan, A. N. Disaccharidase levels in jejunal biopsy specimens from American and South Indian control subjects and patients with tropical sprue. Clin. chim. Acta 30 707, 1970.
21. Weijers, H. A., van de Kamer J H., Dicke, W K. & Ijsseling J Diarrhoea caused by deficiency of sugar splitting enzymes. I. Acta paediat. (Uppsala) 50 55 1961.
22. Yudkin, J Edelman, J & Hough, L (eds) Sugar Butterworths, London 1971

Acta med. scand. Suppl. 542, pp. 19—25

INTESTINAL METABOLISM OF FRUCTOSE

Robert H. Herman, Fred B. Stifel, Harry L. Greene and Yaye F Herman

From the Metabolic and Chemistry Divisions U S Army Medical Research and Nutrition Laboratory Fitzsimons General Hospital Denver Colorado 80240 U.S.A.

Abstract The metabolism f fructose by the small intestine can be analyzed in terms of the following scheme 1) hydrolysis of fructose containing saccharides especially sucrose 2) movement of fructose into the intestinal cell 3) transformation of fructose into glycolytic metabolic intermediates 4) formation of fructose from glucose via sorbitol 5) adaptive regulation of fructose metabolizing enzymes, 6) adaptive responses of other enzymes to fructose. The hydrolysis f sucrose is dependent upon the brush border enzyme sucrase which shows an adaptive response to sucrose diets. The entrance of fructose into the small intestine and the intermediary metabolism of fructose is reviewed Fructose metabolizing enzymes, fructokinase and fructose-1-phosphate aldolase, are regulated by the presence or absence f fructose, folic acid and drugs. Fructose causes adaptive changes in small intestine glycolytic enzymes and decreases the gluconeogenic enzyme fructose-1,6-diphosphatase. Actinomycin D inhibits the adaptive effect of fructose on glycolyti enzymes which suggests that fructose acts via the protein synthetic mechanism

The small intestine is capable of absorbing fructose from the intestinal lumen so that the bulk of the fructose finds its way to the liver A smaller proportion of the fructose is metabolized in the mucosal epithelial cells of the small intestine so that the fructose enters into the glycolytic pathway The metabolism of fructose by the small intestine can be analyzed in terms of the following scheme 1) hydrolysis of fructose containing saccharides, especially sucrose 2) movement of fructose into the intestinal cell 3) transformation of fructose into glycolytic metabolic intermediates 4) formation of fructose from glucose via sorbitol 5) adaptive regulation of fructose metabolizing enzymes 6) adaptive responses of other enzymes to fructose.

The hydrolysis of fructose containing saccharides is a process unique to the small intestine. All of the other processes in the small intestine in many ways are qualitatively similar to the same processes in the liver

Fructose containing saccharides, especially sucrose (7) are hydrolyzed by the disaccharidase sucrase which is found in the brush border surface particles (21). Sucrase shows an adaptive response in both man and rats. Sucrose diets in both man (35) and the rat (3, 8) and fructose in man (35) increase sucrase activity Isocaloric glucose diets also increase intestinal sucrase activity but to a lesser degree than sucrose (37). The adaptive increase of sucrase is a relatively slow

process requiring 2—5 days of sucrose feeding (36) We have postulated that this adaptive increase is due to the effect of the dietary substance on the cells in the germinative crypts. As the cells migrate from the crypts and develop into villus epithelial cells the sucrase activity becomes manifest. The appearance of sucrase activity after institution of a sucrose diet coincides with the turnover time of the villus epithelial cells (36) We have utilized the adaptive effect of fructose to increase the sucrase activity in a child with sucrase deficiency and thus increased her tolerance to exogenous sucrose (11).

Fructose enters into intestinal epithelial cells of man and the rat but not by an active transport mechanism (6 64) The details of fructose movement into intestinal epithelial cells are unknown.

Once in the intestinal epithelial cell fructose is transformed into either fructose-6-phosphate (F-6-P) or fructose-1-phosphate (F 1 P) by the action of hexokinase (52) or fructokinase (1 4 10 47), respectively In the presence of both fructose and glucose as would occur with the hydrolysis of sucrose, fructose is metabolized preferentially by fructokinase since glucose competes with fructose for hexokinase but not for fructokinase (57) After the administration of fructose to the rat there is accumulation of large amounts of F-6-P F 1 P and fructose-1 6-diphosphate (FDP) (24, 25 31).

Fructose-1 phosphate is further transformed into dihydroxyacetone phosphate (DHAP) and glyceraldehyde by means of fructose-1 phosphate aldolase (0 48 49) It has been suggested that this activity is contained in the same enzyme molecule that has fructose-1 6-diphosphate aldolase activity since purification of the liver enzyme has not led to separation of the two activities (19 22, 33). Both F-6-P and DHAP are metabolized via the well known glycolytic pathway By analogy with the events in the liver it is presumed that in the intestine glyceraldehyde is further transformed into phosphoglyceric acid by means of a triose kinase and adenosine triphosphate (ATP) (17) Fructokinase and types I and II hexokinases of the rat and guinea pig jejunums have been purified (63)

In the rat as compared to the guinea pig type II hexokinase predominates and there are only very small amounts of types III and IV hexokinases (63) High concentrations of KCl inhibit rat jejunal hexokinase activity (63). The hexokinases account for at least 30 % of fructose phosphorylation (63)

It has been shown that intestinal hexokinase may exist bound to a particulate fraction (50 53, 54). Perfusion of rat intestine for short periods of time up to 66 mins. has shown an increase in soluble and a decrease in particulate hexokinase. It was concluded that the glucose-dependent increase of hexokinase activity in the soluble cell compartment in the intestinal mucosa of starved rats was due to a release of hexokinase from a particulate subcellular structure (28)

Another system for the phosphorylation of fructose has been found in intestinal mucosa. In guinea pig and rat intestinal mucosa a phosphoenolpyruvate (PEP)-dependent monosaccharide phosphotransferase can transform fructose to F 1 P as contrasted with the ATP-dependent systems in which F 1 P FDP and F-6-P are formed (61) The physiological significance of this PEP-dependent system is not known.

Rat jejunal phosphofructokinase has been isolated and characterized and has been shown to be stabilized by sulfate and inhibited by sodium (20).

Fructose is converted to glucose by rat (21) guinea pig (9, 23) and hamster intestines (63) Guinea pig intestine appears to convert fructose to glucose to a greater degree than rat intestine (23) presumably due to a very low level of glucose-6-phosphatase in rat jejunum (10 18). Fructose is also metabolized to lactate in rats and guinea pigs (23 51).

The accumulation of F 1 P that occurs in the intestine after fructose administration (24

31) may perhaps involve the same mechanisms that occur in the liver The rate of F 1 P formation from fructose and ATP is about the same as the rate of formation of glyceraldehyde and DHAP from F 1 P Thus, it would not be expected that F 1 P would accumulate after fructose administration (66) However such is the case. With fructose metabolism there is a decrease in ATP and inorganic phosphate (29 34). The decrease in organic phosphate levels results in increased activity of adenosinemonophosphate (AMP) deaminase activity since AMP deaminase is inhibited by inorganic phosphate. The increased activity of AMP deaminase results in a decrease in AMP levels and increased formation of inosine monophosphate which inhibits the action of fructose-1-phosphate aldolase (29 34, 66).

In the intestine glucose can be converted into sorbitol by the action of aldose reductase, a high K_m enzyme (5 59). Presumably the sorbitol can form fructose via the action of sorbitol dehydrogenase. The physiological significance of these reactions in the small intestine is unknown

The enzymes which metabolize fructose, fructokinase and fructose-1 phosphate aldolase, can be regulated by the presence or absence of fructose, folic acid and drugs. Thus, adaptive mechanisms exist whereby the activities of these enzymes can be regulated. The term adaptation is used to indicate that a change occurs as a consequence of the action of some agent. The term adaptation should not be taken to mean that any specific mechanism of action is implied. In particular adaptation is not synonymous with the term induction which specifically implies the *de novo* biosynthesis of protein. It may be that some adaptive mechanisms operate via an inductive process but not all adaptive mechanisms necessarily operate in this fashion.

A high fructose diet will cause increased activity of fructokinase and fructose-1 phosphate aldolase in both man (44) and the rat (59). A glucose diet will give lower values of these two enzymes (44, 59). An isocaloric non-carbohydrate diet results in still lower values and fasting gives the lowest values of all (44 59). However the enzyme activities do not disappear but are at their lowest levels with fasting A sucrose diet increased the activities of fructokinase and fructose-1 phosphate aldolase as compared to glucose and carbohydrate-free diets within 24 hours (46). In two fasting obese patients who were fed 50 g each of glucose and fructose the increase in enzyme activities occurred within 6 hours (45). The effect of diet in increasing rat intestinal hexokinase (2, 53, 54, 62) and fructokinase (62) activities has been reported and confirmed by other investigators.

Folic acid is able to increase both fructokinase and fructose-1-phosphate aldolase activities as well as the activities of other glycolytic enzymes (41). Both dietary carbohydrate and folic acid increase the fructose metabolizing enzymes within two hours with the maximum activities seen at 6—12 hours with elevated activities still present at 24 hours (43). With increasing dosages of folic acid there are increasing values of enzyme activities (43). It is hypothesized that the action of dietary carbohydrate and folic acid are direct actions on the villus epithelial cells because of the short time period involved necessary for the increased enzyme activities to occur after the administration of fructose and folic acid (41).

We have speculated that fructose causes enzyme induction, i.e., *de novo* synthesis of enzyme protein while folic acid initiates protein synthesis (14). Inhibitors of protein synthesis block the action of dietary fructose on glycolytic and folate-metabolizing enzymes (58) indicating that the protein synthetic machinery is ultimately involved. The effect of inhibitors of protein synthesis on fructokinase and fructose-1-phosphate aldolase activities was not tested specifically

however. Dietary fructose, however increases glycolytic enzymes and decreases the gluconeogenic enzyme, fructose-1,6-diphosphatase, while folic acid increases all of the glycolytic enzymes and fructose-1,6-diphosphatase. In the scheme of Jacob and Monod (32) the simultaneous induction and repression of enzymes can explain the action of fructose but not the action of folic acid. Folic acid provides two separate cofactors for purine biosynthesis (60) and one might consider that folic acid enhances the biosynthesis of nucleoside triphosphates for messenger RNA synthesis. However there would have to be a corresponding increase in pyrimidine biosynthesis. A folic acid cofactor involves the biosynthesis of deoxyribose thymidine 5' phosphate which is involved in DNA but not RNA synthesis (60). An increased formation of purines could lead to an increase in guanosine triphosphate (GTP) which is intimately involved in protein synthesis at the ribosomal level (26) and in this way might stimulate protein synthesis. We have postulated that the administration of folic acid results in increased formation of [illegible] formyltetrahydrofolic acid which then [illegible] the initiation and hence the forma- [illegible] o the Embden-Meyerhof pathway enzymes (14, 41). It seems clear that formylmethionyl-tetrahydrofolic acid is the initiator o protein biosynthesis in micro-organisms. However this has not yet been proven to be true in mammalian systems. It is of interest that folic acid has no effect on jejunal disaccharidases (41). We thus have to postulate that [illegible]olic acid initiates the synthesis of only some [illegible]oteins in jejunal mucosal cells. In a pa[illegible]nt with jejunal, hepatic and red cell form[illegible]otransferase deficiency that we have [illegible] studying we have found that there is a [illegible]ed failure of jejunal enzyme adaptation to [illegible]tose and folic acid. Formiminotransfera[illegible] [illegible]ransforms tetrahydrofolic acid and formiminoglutamic acid into glutamic acid and formimino[illegible]etrahydrofolic acid which is an intermediate in the pathway leading to N^{10} formyltetrahydrofolic acid. Failure of the adaptive enzyme response in the jejunum of this patient who also has a specific defect in folic acid metabolism is consistent with our hypothesis on the mechanism of action of folic acid (15).

We have utilized the adaptive effect of folic acid to increase the fructose-1-phosphate aldolase activities in the jejunum and liver of a child with hereditary fructose intolerance (12). Coincident with the increase in hepatic and jejunal enzyme activities her hepatomegaly and a fatty infiltrate in the liver both disappeared. Whether the increase in hepatic fructose-1 phosphate aldolase activity was sufficiently large to be of clinical significance, however cannot be stated with certainty.

Orally administered sex hormones (estradiol-17β and testosterone) will increase the activities of several glycolytic enzymes in rat jejunum (56). Male rats respond better to testosterone while female rats respond better to estradiol-17β (56). In these studies the effect of sex hormones on rat fructose metabolizing enzymes was not tested. In normal adult males and hypogonadal male patients we have found no response of fructose-1 phosphate aldolase and fructose-1,6-diphosphate aldolase to oral or intramuscular (hypogonadal patients only) testosterone even though pyruvate kinase activities increased (27). In one female child and her mother who were being studied for other reasons we found a similar lack of response of fructose-1 phosphate and fructose-1 6-diphosphate aldolases to oral estradiol-17β (12). It would appear that the specific fructose metabolizing enzymes of the jejunum are not affected by sex hormones. In rats, folic acid deficiency causes a decrease in jejunal glycolytic enzyme activities (16) and prevents the adaptive increase of enzymes to sex hormones (13).

Fructokinase and fructose-1-phosphate al-

dolase activities have been found to be increased in human jejunum within one day after the administration of phenobarbital, 30 mg, four times per day (42). On the other hand, dexamethasone decreases the activities of these same fructose metabolizing enzymes (38)

Ethanol and folic acid are antagonistic in the jejunum. In man ethanol decreases the activities of jejunal fructose metabolizing enzymes while folic acid increases the activities of the same enzymes. When both are administered together the effect of each is inhibited by the other (40) The mechanism of action of ethanol on fructose metabolizing enzymes is unknown but would seem to be related to the folate effect. It has been known for many years that there is some interaction between folic acid and ethanol but the exact relationship has been unclear The antagonism between folate and ethanol on jejunal fructose metabolizing enzymes brings this relationship more clearly into focus.

Fructose itself can affect various of the glycolytic enzymes. Fructose will increase the activities of rat jejunal fructose-1 6-diphosphate aldolase (39) pyruvate kinase, phosphofructokinase and glycerol-3-phosphate dehydrogenase (55) and decrease fructose-1 6-diphosphatase (35)

Fructose causes similar changes in human jejunal pyruvate kinase, phosphofructokinase and fructose diphosphatase (39) Fructose causes an increase in the activities in rat jejunum of certain of the folic acid metabolizing enzymes (glutamate formiminotransferase, serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase) and a decrease in formyl-tetrahydrofolate synthetase (37) Fructose may cause these adaptive changes in jojunum via the mechanism of depression and repression. Actinomycin D inhibits the adaptive effect of fructose on these enzymes which is consistent with the mechanism of action of fructose via *de novo* protein synthesis (38)

REFERENCES

1. Adelman, R. C., Ballard, F J & Weinhouse, S.. Purification and properties of rat liver fructokinase. J biol. Chem. 242 3360, 1967
2. Anderson, J W & Zakim, D The influence f alloxan diabetes and fasting on glycolytic and gluconeogenic enzyme activities of rat intestinal mucosa and liver Biochim. biophys. Acta 201 236, 1970.
3. Blair D G R., Yakimets, W & Tuba, J Rat intestinal sucrase. II. The effects of rat age and sex and of diet on sucrase activity Can. J Biochem. 41 917 1963.
4. Cadenas, E. & Sols, A. The ketokinase activity of the intestinal mucosa. Biochim. biophys. Acta 42 490, 1960.
5. Clements, R. S., Weaver J P & Winegrad, A. L Th distribution of polyol NADP oxidoreductase in mammalian tissues. Biochem. biophys. Res. Commun. 37 347 1969.
6. Crane, R. K. *Intestinal* absorption of sugars. Physiol. Rev 40 789 1960.
7. Dahlqvist, A.. Specificity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. J clin. Invest. 41 463, 1962.
8. Deren, J J Broitman, S. A. & Zamcheck, N Effect of diet upon *intestinal disaccharidases* and disaccharide absorption. J clin. Invest. 46 186, 1967
9. Fridhandler L. & Quastel, J H. Absorption of sugars from isolated surviving intestine. Arch. Biochem. Biophys. 56 412, 1955.
10. Ginsburg, V & Hers, H. G. On the conversion of fructose to glucose by *guinea pig* intestine. Biochim. biophys. Acta 38 427 1960.
11. Greene, H. L., Stifel, F B. & Herman, R. H. Dietary stimulation of sucrase in patient with sucrase-isomaltase deficiency Lancet I 631 1971
12. Greene, H. L., Stifel, F B. & Herman, R. H.. Hereditary fructose intolerance—treatment with pharmacologic doses of folic acid. Western Soc. Ped. Res Carmel, California, 4—6 F bruary 1972.
13. Herman, R. H., Herman, Y F & Stifel, F B.. Oral folic acid requirement for the normal adaptive response of jejunal pyruvate kinase activity to sex steroids in rats. Fed. Am. Soc. exp. Biol., Atlantic City New Jersey 9—14 April 1972.
14. Herman, R. H. & Rosensweig, N. S The initiation of protein synthesis. Am. J clin. Nutr 22 806, 1969

15 Herman, R. H., Rosensweig, N S., Stifel, F B. & Herman, Y F Adult formiminotransferase deficiency A new entity Clin. Res. 17 304, 1969

16. Herman, R. H., Stifel, F B., Herman, Y F & Rosensweig, N S The response of jejunal glycolytic enzymes to a folate deficient diet in germ-free and pathogen-free rats. Fed. Proc. 28 628, 1969

17 Herman, R. H. & Zakim, D Fructose metabolism. I. The fructose metabolic pathway Am. J clin. Nutr 21 245, 1968.

18. Hers, H. G & de Duve, C. Le systeme hexosephosphatasique. II. Repartition de l activite glucose-6-phosphatasique dans les tissues. Bull. Soc. chim. Biol., (Paris) 32 20, 1950.

19 Hers, H. G & Joassin, G Anomaly of hepatic aldolase in fructose intolerance. Enzymol. biol. Clin. 1 4, 1961

20. Ho W & Anderson, J W Phosphofructokinase in rat jejunal mucosa. Subcellular distribution, isolation, and characterization. Biochim. biophys. Acta 227 354, 1971

21 Johnson, C. F Hamster intestinal brush border surface particles and their function. Fed. Proc. 28 26, 1969

22. Kaletta-Gmünder U Wolf, H. P & Leuthardt, F Aldolases. II. Chromatographic separation of liver 1 phosphofructoaldolase and diphosphofructoaldolase. Helv chim. Acta 40 1027 1957

23. Kiyasu, J Y & Chaikoff, I. L. On the manner of transport of absorbed fructose. J biol. Chem. 224 935, 1957

24. Kjerulf-Jensen, K. Hexose monophosphates formed inside the intestinal mucosa during the absorption of fructose, glucose and galactose. Acta physiol. scand. 4 225 1942.

25. Kjerulf-Jensen, K. The phosphate esters formed in the liver of rats and rabbits during the assimilation of hexoses and glycerol. Acta physiol. scand. 4 249 1942.

26. Lipmann, F Polypeptide chain elongation in protein biosynthesis. Science 164 1024, 1969

27 Lufkin, E. G Stif l F B Herman, R. H., & Rosensweig, N S Effect of testosterone on jejunal pyruvat kinase activities in normal and hypogonadal males J clin Endocr 34 586, 1972

28. Mayer R. J Shakespeare P & Hubscher G Glucose metabolism in the mucosa of the small intestine. Changes of hexokinase activity during perfusion f the proximal half of the rat small intestine Biochem J 116 43, 1970.

29 Mäenpää, P H., Raivio K. O & Kekomäki, M. P Liver adenine nucleotides Fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.

30. Nisell, J & Linden, L. Fructose-1 phosphate aldolase and fructose-1 6-diphosphate aldolase activity in the mucosa of the intestine in hereditary fructose intolerance. Scand. J. Gastroent. 3 80 1968.

31 Papadopoulos, N M. & Roe, J H. Fructose phosphorylation and dephosphorylation by the intestinal mucosa of the rat during fructose absorption. Am. J Physiol. 189 301 1957

32. Pardee, A. B., Jacob, F & Monod, J The genetic control and cytoplasmic expression of Inducibility» in the synthesis of beta-galactosidase by E coli. J mol. Biol. 1 165 1959.

33. Penasky R. J & Lardy H. A. Bovine liver aldolase. I. Isolation, crystallization and some general properties. J biol. Chem. 233 365, 1958.

34 Raivio, K. O Kekomäki, M. P & Mäenpää, P H. Depletion of liver adenine nucleotides induced by D-fructose. Dose dependence and specificity of the fructose effect. Biochem. Pharmacol. 18 2615, 1969

35 Rosensweig, N S. & Herman, R. H. Control of jejunal sucrase and maltase activity by dietary sucrose or fructose in man. J clin. Invest. 47 2253, 1968.

36 Rosensweig, N S. & Herman, R. H. Time response of jejunal sucrase and maltase activity to a high sucrose diet in normal man. Gastroenterology 56 500, 1969

37 Rosensweig, N S. & Herman, R. H. Dose response of jejunal sucrase and maltase activities to isocaloric high and low carbohydrate diets in man. Am. J clin. Nutr 23 1373, 1970.

38 Rosensweig, N S Herman, R. H. & Stifel, F B Effect of dexamethasone, insulin and tolbutamide on human jejunal glycolytic and gluconeogenetic enzyme activities. Fed. Proc. 29 256 1970

39 Rosensweig, N S., Herman, R. H. & Stifel, F B. Dietary regulation of glycolytic enzymes VI. Effect of dietary sugars and oral folic acid on human jejunal pyruvate kinase phosphofructokinase and fructosediphosphatase activities. Biochim. biophys. Acta 208 373, 1970.

40. Rosensweig, N S., Herman, R. H. & Stifel, F B. Ethanol inhibition of the effect of folic acid on jejunal glycolytic enzyme activities Am. J clin. Nutr 23 670, 1970.

41 Rosensweig, N. S Herman, R. H Stifel, F B. & Herman, Y F Regulation of human jejunal glycolytic enzymes by oral folic acid. J clin. Invest. 48 2038, 1969

42. Rosensweig, N. S Stifel, F B. & Herman, R. H. Effect of phenobarbital on human jejunal glycolytic, gluconeogenetic and pentose phosphate path enzymes. Clin. Res. 17 596, 1969

43. Rosensweig, N S., Stifel, F B., Herman, Y F & Herman, R. H. Regulation of human jejunal glycolytic enzymes by oral folic cid Time and dose response. Am. J clin. Nutr 22 667 1969

44. Rosensweig, N S., Stifel, F B., Herman, R. H. & Zakim, D The dietary regulation of the glycolytic enzymes. II. Adaptive changes in human jejunum. Biochim. biophys. Acta 170 228, 1968.

45. Rosensweig, N S., Stifel, F B., Herman, R. H. & Zakim, D Time response of diet induced changes in human jejunal glycolytic enzymes. Fed. Proc. 28 323, 1969

46. Rosensweig, N S., Stifel, F B., Zakim, D. & Herman, R. H. Time response of human *jejunal glycolytic enzymes to high sucrose* diet. Gastroenterology 57 143, 1969

47 Salomon, L. L. & Johnson, J E. Transfer of fructose and glucose across surviving guinea pig intestine. Arch. Biochem Biophys. 82 179 1959

48. Schapira, F The distribution and ctivity of 1-phosphofructoaldolase. Compt. Rend. 247 157 1958.

49 Schapira, F Fructose-1-phosphoaldolase ac tivity of mammalian tissues. I. Its distribution in mammalian tissues. Bull. Soc. chim. Biol. (Paris) 43 1357 1961

50. Shakespeare, P Srivastava, L. M. & Hubscher G Glucose metabolism in the mucosa of the small intestine. The effect f glucose on hexokinase activity Biochem. J 111 63, 1969

51 Sherrat, H. S A. The metabolism of the *small intestine. Oxygen uptake and L-lactat* production along the length of the small intestine of the rat and guinea pig. Comp Biochem. Physiol. 24 745, 1968.

52 Sols, A. Hexokinase activity of the intestinal mucosa. Biochim. biophys. Acta 19 144, 1956.

53 Srivastava, L. M. & Hübscher G Glucose metabolism in the mucosa of the small intestine Glycolysis in subcellular preparations from the cat and rat. Biochem. J 100 458, 1966.

54 Srivastava, L. M Shakespeare, P & Hubscher G Glucose metabolism in the mucosa of the small intestine. A study f hexokinase activity Biochem. J 109 35, 1968.

55. Stif l, F B Herman, R. H. & Rosensweig, N. S. Dietary regulation of glycolytic enzymes. III. Adaptive changes in rat jejunal pyruvate kinase, phosphofructokinase, fructosediphosphatase and glycerol 3-phosphate dehydrogenase. Biochim. biophys. Acta 184 29 1969

56. Stifel, F B Herman, R. H. & Rosensweig, N S. Dietary regulation f glycolytic enzymes IV Differential hormonal effects in male and female rat jejunum. Biochim. biophys. Acta 184 495 1969.

57 Stifel, F B Herman, R. H. & Rosensweig, N S.. Dietary regulation of glycolytic enzymes. VII. Effect of diet and oral folat upon folate-metabolizing enzymes in rat jejunum. Biochim. biophys. Acta 208 381 1970

58. Stifel, F B., Herman, R. H. & Rosensweig, N S.. Dietary regulation of glycolytic enzymes. XI. Effect of inhibitors of protein synthesis on the adaptation of certain jejunal glycolytic and folate-metabolizing enzymes to diet and sex steroids. Biochim. biophys. Acta 237 484, 1971.

59. Stifel, F B., Rosensweig, N S., Zakim, D. & Herman, R. H. Dietary regulation of glycolytic enzymes. I. Adaptive changes in rat jejunum. Biochim. biophys. Acta 170 221, 1968.

60. Stokstad, E. L. R. & Koch, J Folic acid metabolism. Physiol. Rev 47 83, 1967

61. Weiser M. M. & Isselbacher K. J.. Phosphoenolpyruvate-activated phosphorylation of sugars by intestinal mucosa. Biochim. biophys. Acta 208 349, 1970.

62. Weiser M. M., Quill, H. & Isselbacher K. J Effects of diet on rat intestinal soluble hexokinase and fructokinase activities. Am. J Physiol. 221 844, 1971.

63. Weiser M. M., Quill, H. & Isselbacher K. J Isolation and properties of intestinal hexokinases, fructokinase, and N-acetylglucosamine kinase. J biol. Chem. 246 2331, 1971.

64. Wilson, T H. Intestinal Absorption p. 43, Saunders Philadelphia, 1962.

65. Wilson, T H. & Vincent, T N.. Absorption of sugars in vitro by the intestine of the golden hamster J biol. Chem. 216 851, 1955

66. Woods, H. F Eggleston, L. V & Krebs, H. A.. The cause of hepatic accumulation of fructose 1 phosphate on fructose loading. Biochem. J 119 501 1970.

15. Herman, R. H., Rosensweig, N. S., Stifel, F. B. & Herman, Y. F. Adult formiminotransferase deficiency: A new entity. Clin. Res. 17, 304, 1969.
16. Herman, R. H., Stifel, F. B., Herman, Y. F. & Rosensweig, N. S. The response of jejunal glycolytic enzymes to a folate deficient diet in germ-free and pathogen-free rats. Fed. Proc. 28, 628, 1969.
17. Herman, R. H. & Zakim, D. Fructose metabolism. I. The fructose metabolic pathway. Am. J. clin. Nutr. 21, 45, 1968.
18. Hers, H. G. & de Duve, C. Le système hexosephosphatasique. II. Répartition de l'activité glucose-6-phosphatasique dans les tissues. Bull. Soc. chim. Biol., (Paris) 32, 20, 1950.
19. Hers, H. G. & Joassin, G. Anomaly of hepatic aldolase in fructose intolerance. Enzymol. biol. Clin. 1, 4, 1961.
20. Ho, W. & Anderson, J. W. Phosphofructokinase in rat jejunal mucosa. Subcellular distribution, isolation, and characterization. Biochim. biophys. Acta 227, 354, 1971.
21. Johnson, C. F. Hamster intestinal brush border surface particles and their function. Fed. Proc. 28, 26, 1969.
22. Kaletta-Gmünder, U., Wolf, H. P. & Leuthardt, F. Aldolases. II. Chromatographic separation of liver 1-phosphofructoaldolase and diphosphofructoaldolase. Hel. chim. Acta 40, 1027, 1957.
23. Kiyasu, J. Y. & Chaikoff, I. L. On the manner of transport of absorbed fructose. J. biol. Chem. 224, 935, 1957.
24. Kjerulf-Jensen, K. Hexose monophosphates formed inside the intestinal mucosa during the absorption of fructose, glucose and galactose. Acta physiol. scand. 4, 225, 1942.
25. *Kjerulf-Jensen, K. Th[illegible] phosphat esters* formed in the liver [illegible] and rabbits during the assimilation [illegible] nd glycerol. Acta physiol. scand. 4 [illegible]
26. Lipmann, F. Pol[illegible] longation in protein biosynthes[illegible] 164, 1024, 1969.
27. Lufkin, E. G., Stif[illegible] H[illegible]rman, R. H., & Rosensweig, N[illegible] testosterone on jejunal pyru[illegible] [illegible]ti[illegible]es in normal and hyp[illegible] J. [illegible] Endocr. 34, 586, 19[illegible]
28. Mayer, R. J., Shake[illegible] H[illegible] her, G. Glucose metabolism [illegible] the small intestine. Chan[illegible] activ[illegible]ity during perfusion [illegible] half of the rat small intes[illegible] J. 116, 43, 1970.
29. Maenpää, P. H., Raivio, K. O. & Kekomäki, M. P. Liver adenine nucleotides: Fructose-induced depletion and its effect on protein synthesis. Science 161, 1253, 1968.
30. Nisell, J. & Linden, L. Fructose-1-phosphate aldolase and fructose-1,6-diphosphate aldolase activity in the mucosa of the intestine in hereditary fructose intolerance. Scand. J. Gastroent. 3, 80, 1968.
31. Papadopoulos, N. M. & Roe, J. H. Fructose phosphorylation and dephosphorylation by the intestinal mucosa of the rat during fructose absorption. Am. J. Physiol. 189, 301, 1957.
32. Pardee, A. B., Jacob, F. & Monod, J. The genetic control and cytoplasmic expression of inducibility in the synthesis of beta-galactosidase by *E. coli*. J. mol. Biol. 1, 165, 1959.
33. Penhosky, R. J. & Lardy, H. A. Bovine liver aldolase. I. Isolation, crystallization and some general properties. J. biol. Chem. 233, 365, 1958.
34. Raivio, K. O., Kekomäki, M. P. & Mäenpää, P. H. Depletion of liver adenine nucleotides induced by D-fructose. Dose dependence and specificity of the fructose effect. Biochem. Pharmacol. 18, 2615, 1969.
35. Rosensweig, N. S. & Herman, R. H. Contr[illegible] of jejunal sucrase and maltase activity [illegible] dietary sucrose or fructose in man. J. cl[illegible] Invest. 47, 2253, 1968.
36. Rosensweig, N. S. & Herman, R. H. The response of jejunal sucrase and maltase a[illegible]tivity to a high sucrose diet in normal ma[illegible] Gastroenterology 56, 500, 1969.
37. Rosensweig, N. S. & Herman, R. H. Dos[illegible] response of jejunal sucrase and maltase ac[illegible]tivities to isocaloric high and low carbohydrate diets in man. Am. J. clin. Nutr. 2[illegible] 1372, 1970.
38. Rosensweig, N. S., Herman, R. H. & Stifel, F. B. Effect of dexamethasone, insulin and tolbutamide on human jejunal glycolytic and gluconeogenetic enzyme activities. Fed. Proc. 29, 258, 1970.
39. Rosensweig, N. S., Herman, R. H. & Stifel, F. B. Dietary regulation of glycolytic enzymes VI. Effect of dietary sugars and oral folic acid on human jejunal pyruvate kinase, phosphofructokinase and fructosediphosphatase activities. Biochim. biophys. Acta 2[illegible] 373, 1970.
40. Rosensweig, N. S., Herman, R. H. & Stifel, F. B. Ethanol inhibition of the effect of folic acid on jejunal glycolytic enzyme activities. Am. J. clin. Nutr. 23, 670, 1970.

Acta med. scand. Suppl. 542, pp. 27—36

METABOLISM OF FRUCTOSE IN LIVER

Fritz Heinz

From the Department of Biochemistry Medizinische Hochschule Hannover

Abstract For liver metabolic pathway for fructose has been described in which the ketosugar is phosphorylated by ketohexokinase to fructose-1 phosphate, which is then converted by liver aldolase to D-glyceraldehyde and dihydroxyacetone phosphate, an intermediate of the glycolytic pathway D-glyceraldehyde could be oxidized to glycerate. By phosphorylation glycerate becomes an intermediate of the Embden Meyerhof pathway like D-glyceraldehyde if phosphorylated directly by triokinase. The reduction f D-glyceraldehyde forming glycerol, which could be phosphorylated to L-glycerol 3-phosphate is in variance with isotope studies with fructose-6-^{14}C.

This special metabolic pathway is limited to warmblooded animals and man, because only in the liver of these species ketohexokinase could be detected.

An adaption f enzymes was found in rats, which have had a high fructose diet over three weeks.

The activity of ketohexokinase estimated under optimal conditions and 37°C agrees well with fructose extraction rates found in liver perfusion for rat and in in vivo experiments for human liver The fast catabolism f fructose in human liver in contrast to glucose, is due to higher enzyme levels f ketohexokinase, in contrast to hexokinase and glucokinase. By this high phosphorylation capacity and the low activity f aldolase together with the action f metabolic inhibitors on this enzyme and the equilibrium fa t the side of fructose-1 phosphate, fructose-1-phosphate will accumulate if high fructose concentrations were offered. But even under these conditions, the levels f metabolites following fructose-1 phosphate were enlarged.

Enzyme regulations based on the high fructose-1 phosphate and low ATP levels were discussed.

In 1874, Külz (27) reported that fructose, in contrast to glucose, is tolerated by human diabetics. Twenty years later first experiments with pancreatectomized dogs were described by Minkowski (34). According to this author glycogen was synthesized in the liver of animals fed on fructose but not on glucose. In 1902, Oppenheimer (37) found in dog livers perfused *in vitro* that the administration of fructose to the perfusion fluid, in contrast to glucose, resulted in a higher production of lactate. Similar results were obtained by Embden and Isaac (7) These authors found that the biosynthesis of lactate from fructose remained unchanged in perfused livers of diabetic dogs no lactate was produced by the administration of glucose.

Accordingly the early biochemists trying to get more information about carbohydrate

metabolism, recognized the differences between sugars as glucose and fructose In 1950—1952, Leuthardt and Testa (32 a, b), Cori et al. (4), and Hers (18) found an ATP dependent enzyme in liver phosphorylating fructose to fructose-1 phosphate. In the newer literature this enzyme was called ketokinase or ketohexokinase Recently this enzyme was purified from rat liver by Sanchez et al. (42) potassium and magnesium were needed in order to obtain optimal activity Fructose-1 phosphate is converted to dihydroxyacetone phosphate and D-glyceraldehyde by aldolase 1 B which is present only in the liver (8 a, b 25 38) The properties of the enzyme were different from those of the muscle enzyme. Dikow et al. (6) succeeded in obtaining a pure enzyme preparation from the human liver Dihydroxyacetone phosphate may enter the glycolytic path way Metabolism of the D-glyceraldehyde however can be catalyzed by four different enzymes.

The triokinase catalyzes the phosphory n of D-glyceraldehyde using ATP to -glyceraldehyde-3-phosphate (13 20 21 22). The aldehyde dehydrogenase (NAD) oxidizes D-glyceraldehyde to glyceric acid (23, 29 39) The latter compound is again phosphorylated via the glycerate kinase and ATP to 2 phosphoglycerate an intermediate of the Embd n Meyerhof pathway (28 30). This metabol c pathway was first discussed by Leu hardt et al (33) In addition, the formation of serin is also possible by the NAD / NADP -dependent dehydrogenisation of D-glycerate to hydroxypyruvate (11 47) A reduction of the glyceraldehyde to glycerol can occur by the action of two different alcohol dehydrogenases The so-called normal alcohol dehydrogenase (24 48) requires NADH the other one requires NADPH and was formerly named aldehyde reductase or glycerol dehydrogenase Thus glycerol is phosphorylated by the glycerol kinase to L-glycerol-3-phosphate (1 46) a compound belonging to the glycolytic pathway The reduction of the D-glyceraldehyde to glycerol is not consistent with isotope studies using fructose-6-^{14}C (19 41) If this labelled ketohexose is administered to rats, a more pronounced radioactivity is found in the carbon atoms number one and six of glucose derived from the hepatic glycogen. Glycerol formed by the reduction of D-glyceraldehyde-3-^{14}C derived from fructose-6-^{14}C, is phosphorylated stereospecifically to L-glycerol-3-phosphate-1 ^{14}C via the glycerol kinase reaction (2) This observation is in agreement with the Three Point Attachment Theory» according to Ogston (36)

L-glycerol-3-phosphate-1 ^{14}C introduces the label to the carbon atoms number 3 and 4 of the glucose molecule. However this labelling was not detectable in the hepatic glucose from animals treated with fructose-6-^{14}C. Besides the K_m of 3×10^{-2} M of the liver alcohol dehydrogenase using glyceraldehyde is too high with respect to physiological processes.

These possibilities concerning fructose metabolism and its intermediates — as shown in Fig 1 — do not give any answer to the following questions

1 Fructose metabolism, is it a common pathway in the liver of all animals and in man?
2. The fructolytic enzymes, are they localized only in the liver? Are these enzymes definitely involved in fructose metabolism? The enzyme levels, are they high enough in order to explain the well known high metabolic rate of fructose in the liver and even so in contrast to glucose?
3. The application of fructose, does it change the concentrations of metabolic intermediates of fructose and glucose? What is the magnitude of these changes?

METHODS

Enzymes were extracted from liver and measured as was previously described (14). D-glyceraldehyde was used as a substrate for aldehyde

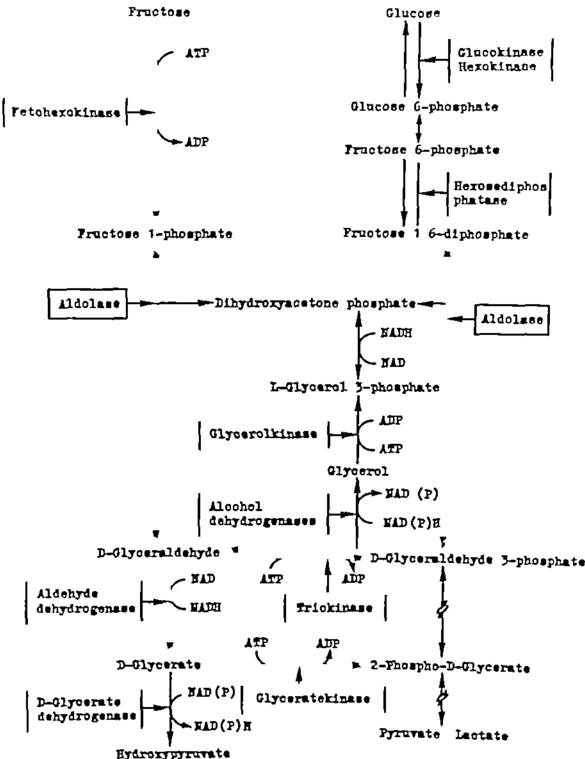


Fig 1 The metabolism f fructose in liver

metabolism, recognized the differences between sugars as glucose and fructose. In 1950—1952, Leuthardt and Testa (32 a, b), Cori et al. (4), and Hers (18) found an ATP dependent enzyme in liver phosphorylating fructose to fructose-1-phosphate. In the newer literature this enzyme was called ketokinase or ketohexokinase. Recently this enzyme was purified from rat liver by Sanchez et al. (42) potassium and magnesium were needed in order to obtain optimal activity Fructose-1 phosphate is converted to dihydroxyacetone phosphate and D-glyceraldehyde by aldolase 1 B which is present only in the liver (8 a, b, 25 38) The properties of the enzyme were different from those of the muscle enzyme. Dikow et al. (6) succeeded in obtaining a pure enzyme preparation from the human liver Dihydroxyacetone phosphate may enter the glycolytic pathway Metabolism of the D-glyceraldehyde, however can be catalyzed by four different enzymes.

The triokinase catalyzes the phosphorylation of D-glyceraldehyde using ATP to glyceraldehyde-3-phosphate (13, 20 21, 22). he aldehyde dehydrogenase (NAD^+) oxidizes D-glyceraldehyde to glyceric acid (23 29 39). The latter compound is again phosphorylated via the glycerate kinase and ATP to 2 phosphoglycerate, an intermediate of the Embden-Meyerhof pathway (28, 30) This ... pathway was first discussed by ... rardt et al (33) In addition, the formation of serin is also possible by the NAD^+/ ADP -dependent dehydrogenisation of D-gl cerate to hydroxypyruvate (11 47). A reduction of the glyceraldehyde to glycerol can occur by the action of two different alcohol dehydrogenases The so-called normal alcohol dehydrogenase (24, 48) requires NADH, the other one requires NADPH and was formerly named aldehyde reductase or glycerol dehydrogenase This glycerol is phosphorylated by the glycerol kinase to L-glycerol-3-phosphate (1 46), a compound belonging to the glycolytic pathway The reduction of the D-glyceraldehyde to glycerol is not consistent with isotope studies using fructose-6-^{14}C (19 41) If this labelled ketohexose is administered to rats, a more pronounced radioactivity is found in the carbon atoms number one and six of glucose derived from the hepatic glycogen. Glycerol formed by the reduction of D-glyceraldehyde-3-^{14}C derived from fructose-6-^{14}C, is phosphorylated stereospecifically to L-glycerol-3-phosphate-1 ^{14}C via the glycerol kinase reaction (2). This observation is in agreement with the Three Point Attachment Theory according to Ogston (36)

L-glycerol 3-phosphate-1 ^{14}C introduces the label to the carbon atoms number 3 and 4 of the glucose molecule. However this labelling was not detectable in the hepatic glucose from animals treated with fructose-6-^{14}C. Besides the K_m of 3×10^{-2} M of the liver alcohol dehydrogenase using glyceraldehyde, is too high with respect to physiological processes.

These possibilities concerning fructose metabolism and its intermediates — as shown in Fig. 1 — do not give any answer to the following questions

1 Fructose metabolism, is it a common pathway in the liver of all animals and in man?
2 The fructolytic enzymes, are they localized only in the liver? Are these enzymes definitely involved in fructose metabolism? The enzyme levels, are they high enough in order to explain the well known high metabolic rate of fructose in the liver and even so in contrast to glucose?
3 The application of fructose, does it change the concentrations of metabolic intermediates of fructose and glucose? What is the magnitude of these changes?

METHODS

Enzymes were extracted from liver and measured as was previously described (14). D-glyceraldehyde was used as a substrate for aldehyde

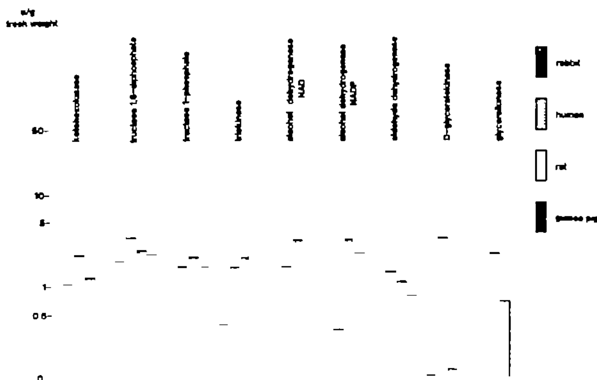


Fig. 3. Activities of fructolytic enzymes in human and laboratory animal liver

ase glycerate kinase and triokinase, as compared to animals fed on an isocaloric diet containing glucose. The triokinase was most elevated. These differences were also seen if animals were fed on a normal diet. The ketohexokinase was found to be the only exception. These results underlined that the triokinase converting the D-glyceraldehyde to D-glyceraldehyde-3-phosphate is definitely involved in fructose metabolism.

The enzyme levels were determined under optimal conditions and at 25 C. If the activity of the ketohexokinase is transformed to 37 C, the physiological temperature of rats, 3.0 u/g (fresh weight) were found. This value is consistent with the rate of fructose extraction from the perfusion fluid using the perfusion method according to Schimassek (43). In human liver the ketohexokinase activity is raised to 1.7 u/g at 37 C. This result is in good agreement with the extraction rate of 1.5—2.3 μmoles fructose/g/min calculated from data obtained in *in vivo* experiments, as was reported by Tygstrup et al. (45) and by Craig et al. (5). It is concluded from these results that fructose is phosphorylated by the ketohexokinase with an optimal rate if the fructose concentration exceeds 20 mg /a.

In rat liver the phosphorylating capacity for glucose is 1.6 μmoles/g/min. This value is similar to ketohexokinase however 1.35 u/g correspond to glucokinase with a high K_m of 1×10^{-2} M and 0.25 u/g correspond to the hexokinase isoenzymes with low K_m values approximating 10^{-5} M. Under physiological conditions with glucose concentrations of 90 mg % in the blood and in the liver only the hexokinase is saturated with this substrate and operates under optimal conditions. The glucokinase phosphorylates about 0.2 μmoles/g/min. We assume that

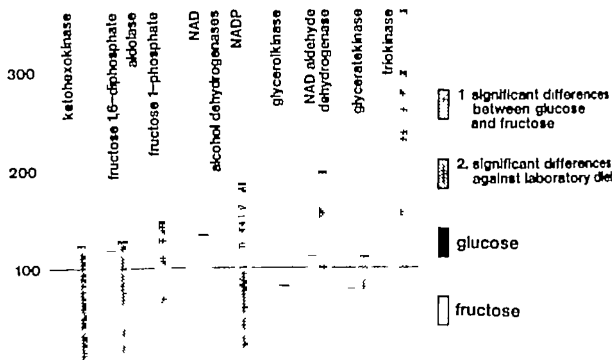


Fig 4 Changes of activities of enzymes in olved in fructose metabolism after feeding a diet containing 60 / fructose or glucose during 3 weeks. Normal diet = 100 /s.

these data may explain the observation that in the liver a higher phosphorylating capac ity was found for fructose, as compared to glucose. This is more pronounced in the human liver because only traces of glucokinase activity were measured.

By the administration of fructose to rats (75 mg/100 g body weight) by a single and brief injection into the tail vein, the fructose concentration in the blood was increased to 10 μmoles per ml (12) Under experimental conditions, the concentration of fructose-1 phosphate in the liver was as high as 7 μmoles/g fresh weight (Fig 5) The content of ATP is reduced by this high phosphoryla tion rate (40) and in order to phosphorylate again the ADP the concentration of inorganic phosphate is diminished in the blood (49) The levels of other intermediates as dihydroxyacetone phosphate fructose 1 6-di phosphate, lactate pyruvate and L-glycerol-3-phosphate were also increased but not as drastically as fructose 1 phosphate. As was demonstrated by Kupke et al. (26), L-glycerol-3-phosphate derived from fructose is easily incorporated into the liver lipids. Therefore, *an augmented supply of this lipid precursor* may stimulate lipid synthesis. Our results on fructose intermediates following fructose loading are comparable to those reported by Burch et al. (3)

The accumulation of fructose-1 phosphate may be due to the relative low aldolase activity and its inhibition by fructose-1,6-diphosphate The latter compound as an intermediate of the Embden Meyerhof pathway is split by the same enzyme. These two criteria and the equilibrium constant between fructose-1 phosphate and dihydroxyacetone phosphate and D-glyceraldehyde which is



Fig. 5 Comparison of ketohexokinase activity with the extraction rate of fructose from the perfusion media of rat liver and the extraction rate of human liver in vivo experiments.

shifted to about 99 % in direction of fructose-1-phosphate probably lead to an elevation of the fructose-1-phosphate content (31). Besides the accumulation of this sugar phosphate may also be the result of a competitive inhibition of the aldolase by degradation products of the ATP metabolism, as was described by Woods et al. (50). Gluconeogenesis from fructose starting after a lack period when the fructose-1-phosphate concentration decreases, can be inhibited by this product at the stage of the glucose-6-phosphate isomerase (31). Obviously the marked decrease of the ATP concentration and the ratio ATP/ADP (40) during the first phase

Fig. 6 Levels of some metabolites in rat liver after single and brief injection into the tail vein (75 mg fructose per 100 g body weight). (fru 1 P = fructose-1 phosphate, DAP = dihydroxyacetone phosphate, fru 1,6-p2 = fructose-1,6-diphosphate, lac = lactate, pyr = pyruvate and L-gly 3-p = L-glycerol-3-phosphate. Quotient = ratio lactate/pyruvate.)

following the application of fructose, changes biochemical reactions requiring energy As was demonstrated by Söling (44) the administration of fructose to rats caused a transformation of the pyruvate dehydrogenase to the active dephospho-enzyme in the liver This activation of the enzyme and the elevated pyruvate concentration increases the acetyl-CoA level as was reported by Helmreich et al. (17). In addition, the formation of the phosphorylase a — the active form — requires ATP therefore, the rate of glycogenolysis may be inhibited. Simultaneously glycogenolysis may be impaired by a diminished supply of inorganic phosphate (49). The system synthesizing glycogen appears to be more complicated. It should be expected that a low ATP concentration would lead to an enhanced conversion of the glycogen synthetase b, the inactive form, to the glycogen synthetase a, the active form. The previous step, however the condensation of the glucose-1-phosphate with UTP forming UDP glucose, depends on a sufficient energy supply within the cell. The reason is that the rephosphorylation of UDP as a product of the glycogen synthetase reaction is coupled to the ATP system.

ACKNOWLEDGEMENT

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Bublitz, C & Kennedy E P J Synthesis of phosphatides in isolated mitochondria III. The enzymatic phosphorylation of glycerol. J biol. Chem. 211 951, 1954
2. Bublitz, C. & Kennedy E P J A note on the asymmetrical metabolism of glycerol. J biol. Chem. 211 963 1955
3. Burch, H. B Lowry H. O Meinhardt, L., Max, P Jr & Fyung J Chyu Effect of fructose, dihydroxy cetone, glycerol, and glucose on metabolites and related compounds in liver and kidney J biol. Chem. 245 2029 1970.
4. Cori, G T Ochoa, S., Slein, M. W & Cori, C. F.. The metabolism of fructose in liver. Isolation of fructose-1-phosphate and inorganic pyrophosphate. Biochim. biophys. Acta 7 304, 1951.
5. Craig, J W Drucker W R., Miller, M, Owens, J E., Woodward, H. jr Brofman, B. & Pritchard, W H. Metabolism of fructose by liver of diabetic and non-diabetic subjects Proc. Soc. expt. Biol. Med. 78 698, 1951.
6 Dikow A. L., Jeckel, D & Pfleiderer G. Isolierung und Charakterisierung von Aldolase A, B und C aus menschlichen Organen. Z. physiol. Chem. 352 1151 1971
7 Embden, G & Isaak, S Über die Bildung von Milchsäure und Acetessigsäure in der diabetischen Leber Z. physiol. Chem. 99 297, 1917
8 a. Göschke, H. & Leuthardt, F Crystallisation of an aldolase from dog liver Helv chim. Acta 46 1791 1963
8 b. Göschke, H. & Leuthardt, F Preparation of pure aldolase from rabbit liver Helv physiol. pharmacol. Acta 21 C 18 1963.
9 Haeckel, R. & Heinz, F.. Unpublished data
10 Heinz, F.. Enzyme des Fructosestoffwechsels Änderungen von Enzymaktivitäten in Leber und Niere der Ratte bei fructose- und glucosereicher Ernährung. Z. physiol. Chem. 349 399 1968
11. Heinz, F Bartelsen, K. & Lamprecht, W D-Glycerat Dehydrogenase aus Leber Beitrag zum Serinstoffwechsel. Z. physiol. Chem. 329 222, 1962.
12. Heinz, F & Junghänel, J Metabolitmuster in Rattenleber nach Fructoseapplikation. Z physiol. Chem. 350 859 1969.
13. Heinz, F & Lamprecht, W Anreicherung und Charakterisierung einer Triokinase aus Leber Zur Biochemie des Fructosestoffwechsels III. Z. physiol. Chem. 324 88, 1961
14. Heinz, F & Lamprecht, W.. Enzyme des Fructosestoffwechsels Aktivität und Verteilung in der Leber der Ratte. Z. physiol. Chem. 348 855 1967
15. Heinz, F Lamprecht, W & Kirsch, J Enzymes of fructose metabolism in human liver J clin. Invest. 47 1826, 1968.
16. Heinz, F & Weiner F.. Enzymes of fructose metabolism in liver of some vertebrates. Comp. biochem. Physiol. 31 283, 1969.
17 Helmreich, E., Goldschmidt, St., Lamprecht W & Ritzl, F Der Einfluß von Kohle-

hydraten, insbesondere von Fructose, uf den Umfang und den zeitlichen Ablauf der Bildung von aktivierter Essigsaure und Brenztraubensäure in der Rattenleber II. Vergleichende Untersuchungen über die Dissimilation der Fructose und der Glucose. Z. physiol. Chem. 292 164, 1953.
18. Hers, H. G La fructokinase du foie. Biochim. biophys. Acta. 8 416 1952.
19. Hers, H. G The conversion of fructose-1-^{14}C and sorbitol-1-^{14}C to liver and muscle glycogen in the rat. J biol. Chem. 214 373, 1955.
20 Hers, H. G Presence of sorbitol in seminal vesicles in foetal blood of the sheep. Biochem. J 66 20 1957
21 Hers, H. G Le Métabolisme du Fructose, Editions Arscia, Bruxelles. 48, 1957
22. Hers, H. G & Kusaka, T Le métabolisme du fructose-1-phosphate dans l foie. Biochim. biophys. Acta 11 427 1953.
23. Holldorf, A., Holldorf, C., Schneider G & Holzer H. Aldehyd Dehydrogenase aus Leber ein Enzym des Fructosestoffwechsels. Z. Naturfrsch. 14 b 229 1959
24. Holzer H. & Schneider S Zum Mechanismus der Beeinflussung des Alkoholoxydation in der Leber durch Fructose. Klin. Wschr. 33 1006, 1955.
25. Kaletta-Gmünder U Wolf, H. P & Leuthardt, F Chromatographische Trennung von 1 Phosphofructaldolase und Diphosphofructaldolase der Leber Helv chim. Acta 40 1027 1957
26. Kupke, I. & Lamprecht, W Über die Biosynthese von Lipiden aus Fructose in Leber II. Einbau von spezifisch markierter ^{14}C Fructose in Leberlipide. Z. physiol. Chem. 348 929 1967
27 Külz, E. Beitrag zur Pathologie und Therapie des Diabetes mellitus. Marburg 1874.
28. Lamprecht, W Diamantstein, T Heinz, F & Balde, P Phosphorylierung von D-Glycerinsäure zu 2 Phospho-D-Glycerinsäure mit Glyceratkinase in Leber I. Zur Biochemie des Fructosestoffwechsels II. Z. physiol. Chem. 316 97 1960.
29 Lamprecht, W & Heinz, F.. Isolierung von Glycerinaldehyd-Dehydrogenase aus Rattenleber Zur Biochemie des Fructosestoffwechsels I. Z. Naturfrsch. 13 b 464, 1958.
30. Lamprecht, W Heinz, F & Diamantstein, T Phosphorylierung von D-Glycerinsäure zu 2 Phospho-D-Glycerinsäure mit Glyceratkinase in Leber II. Identifizierung des Reaktionsproduktes durch Papierchromatographie. Z. physiol. Chem. 328 204, 1962.
31. Leuthardt, F Aldolasen. Handbuch der physiolog. und patholog.-chem. Analyse. 6 C 607 1966.
32 a. Leuthardt, F & Testa, E. Di Phosphorylierung der Fructose in der Leber Helv chim. Acta 34 931, 1951.
32 b. Leuthardt, F & Testa, E.. Über die Phosphorylierung der Ketosen. Helv physiol. pharmacol. Acta 8 67 1950
33. Leuthardt, F Testa, E. & Wolf, H. P.. Der enzymatische Abbau des Fructose-1-phosphats in der Leber Helv chim. Acta 36 227 1953.
34. Minkowski, O.. Untersuchungen über den Diabetes mellitus nach Exstirpation des Pankreas. Arch. exper path. Pharmak. 31 85/189, 1893.
35. Moore, B W A TPN specific glycerol dehydrogenase from liver J Am. chem. Soc. 81 5873, 1959
36. Ogston, A. G.. Conditions for differential reaction of identical chemical groups. Nature 181 1462, 1958
37 Oppenheimer S. Über die Milchsäurebildung in der künstlich durchströmten Leber Biochem. Zschr 45 30, 1902.
38. Peanasky R. J & Lardy H. A.. Bovine liver aldolase. I. Isolation, crystallization and some general properties. J biol. Chem. 232 365, 1958.
39. Racker E. Aldehyde dehydrogenase, di phosphopyridine nucleotide-linked enzyme. J biol. Chem. 177 883, 1949
40. Raivio, K. O Kekomäki, M. P & Mäenpää, P H. Depletion of liver adenine nucleotides induced by D-fructose. Dose-dependence and specificity of the fructose effect. Biochem. Pharmacol. 18 2615, 1969
41 Rauschenbach, P & Lamprecht, W.. Einbau von ^{14}C-markierter Glucose und Fructose in Leberglykogen. Zum Fructosestoffwechsel in der Leber Z. physiol. Chem. 339 277 1964.
42. Sánchez, J J González, N. S. & Pontis, H. G Fructokinase from rat liver I. Purification and properties. II. The role of K+ on the enzyme activity Biochim. biophys. Acta 227 67 79, 1971.
43. Schimassek, H.. Metabolite des Kohlenhydratstoffwechsels der isoliert perfundierten Rattenleber Biochem. Z. 336 460, 1963.
44. Söling, H. D & Bernhard, G.. Interconversion of inactive to active pyruvate dehydrogenase in rat liver after fructose application in vivo. FEBS Letters 13 201, 1967

45. Tygstrup, N Winkler K. & Lundquist, F The mechanism of the fructose effect on the ethanol metabolism of the human liver J clin. Invest. 44 817 1965
46. Wieland, O & Suyter M. Glycerokinase. Isolierung und Eigenschaften des Enzyms. Biochem. Z. 329 320, 1957
47 Willis, J E. & Sallach, H. J Evidence for a mammalian D-glyceric dehydrogenase. J biol. Chem. 237 910 1962.
48. Wolf, H. P & Leuthardt, F Über die Glycerindehydrogenase aus Leber Helv chim. Acta 36 1463, 1953.
49 Wolf, H. P Queisser W & Beck, K. Der initiale Phosphatabfall im Serum von Gesunden und Leberkranken nach intravenöser Verabreichung von Hexosen und Zuckeralkoholen. Klin. Wschr 47 1048, 1969
50 Woods, H. F Eggleston, L. K. & Krebs, H. A. The cause of hepatic accumulation of fructose 1 phosphate on fructose loading Biochem. J 119 501 1970
51 Zalitis, J & Oliver I. T Inhibition of glucose phosphate isomerase by metabolic intermediates of fructose. Biochem. J 102 753, 1962.

Acta med. scand. Suppl. 542, pp. 37—46

FRUCTOSE METABOLISM IN ADIPOSE TISSUE

E. R. Froesch

From the Metabolic Unit Department of Medicine University of Zurich, Switzerland

Abstract. The rate limiting step of fructose metabolism in dipose tissue is fructose transport into the cell. The step is accelerated by insulin only in the absence of glucose, so that insulin has no effect on fructose transport under physiologic conditions. The fructose carrier has relatively high apparent K_m for fructose transport so that significant quantities are transported only t relatively high concentrations of fructose in the blood.

Fructose is the phosphorylated to fructose-6-phosphate by the enzyme hexokinase. Glucose does not compet because it is not present intra cellularly in sufficiently high concentrations. This statement is correct only for adipose tissue.

Fructose uptake by adipose tissue of diabetic animals is reduced as is glucose uptake but to lesser extent.

In vivo, most of the C^{14}-incorporation of fructose-C^{14} into triglycerides or $C^{14}O_2$ by dipose tissue occurs after hepatic conversion of C^{14} fructose to C^{14}-glucose. Insulin is required for glucose transport into adipose tissue.

The main pathway of fructose is its phosphorylation by fructokinase in the liver small bowel and kidney to fructose-1-phosphate, the splitting of fructose-1 phosphate by the B-aldolase and the subsequent phosphorylation of glyceraldehyde by triosekinase (for review see 5). As we will hear in other papers during this Symposium, fructose phosphorylation occurs very rapidly and the half life of fructose in man is approximately 18 minutes. The utilization of fructose by other tissues has been a matter of controversy for many years. There can be no doubt that fructose can, indeed, be metabolized by some tissues other than liver small bowel and kidney Thus, patients with hereditary fructose intolerance who lack the specific hepatic fructose pathway excrete only between 10—20 % of the intravenously administered fructose in the urine (8). The same holds true for patients with essential fructosuria who are deficient in fructokinase (16) the first enzyme of the specific hepatic fructose pathway Therefore, some tissues of the body must be able to metabolize fructose by other metabolic routes.

THE IMPORTANCE OF FRUCTOSE TRANSPORT INTO VARIOUS TISSUES FOR ITS METABOLISM

Hexokinase of many tissues phosphorylates fructose at a rate equal to that of glucose. The main difference in the enzyme reaction towards glucose and fructose lies in the K_m of the reaction. Hexokinase of adipose tissue phosphorylates glucose with a K_m between 10^{-4} and 10^{-5} M, whereas fructose is phosphorylated with a K_m between 10^{-3} and 10^{-2} M (3 10)

Let us consider as a model cells in which glucose and fructose concentrations equilibrate rapidly with the surrounding medium, such as erythrocytes and leucocytes. Red and white blood cells metabolize fructose and glucose to approximately the same extent at concentrations around 200 mg % in the incubation medium when either one of these sugars is present in the medium. Glucose oxidation is not influenced by the addition of cold fructose to the medium, which is to be expected since the K_m of hexokinase is much lower towards glucose than towards fructose. However if one looks at $^{14}CO_2$ formation from ^{14}C fructose an inhibition of this process is observed upon adding cold glucose to the incubation medium (Fig 1) It is, therefore, to be expected that all tissues and all cells devoid of fructokinase which contain free glucose within the cytoplasm do not metabolize fructose although fructose may be transported into the cell because the hexokinase reaction towards fructose is competitively inhibited by glucose. Cells in the body which contain free glucose within the cytoplasm are usually insulin independent with regard to glucose transport. This is certainly true for the above mentioned cells in blood and for the brain and also for liver small bowel mucosa, proximal tubular endothelial cells and for blood vessels. It is therefore, to be expected that red and white blood cells and brain cells do not utilize fructose under normal circumstances, whereas the other mentioned cells utilize fructose by way of the specific fructose pathway rather than by phosphorylation through hexokinase.

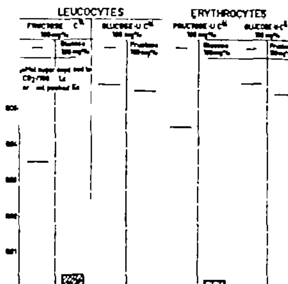


Fig. 1 Fructose and glucose metabolism of leucocytes and erythrocytes. Erythrocytes were separated from leucocytes and incubated in a medium containing ^{14}C-fructose alone or with cold glucose in one set of experiments and ^{14}C glucose alone or with cold fructose in another set of experiments. $^{14}CO_2$ production was measured. Fructose and glucose metabolism by these cells are similar when only the labelled sugar is present. Cold glucose drastically decreases ^{14}C-fructose oxidation, whereas cold fructose has no effect on ^{14}C-glucose oxidation. (From ref. 8)

The question whether the muscle utilizes fructose or not is still a matter of debate. Again, in *in vitro* experiments, the muscle takes up fructose readily incorporating it into glycogen and oxidizing it to CO_2 (Fig 2) (4) However when cold glucose is added in increasing amounts to the incubation medium, fructose utilization by muscle decreases. At a concentration of 200 mg % of glucose and in the presence of insulin, fructose utilization is almost completely abolished. This would tend to indicate that there is no free glucose present in the cytoplasm of the cell at concentrations of glucose below 50 to 100

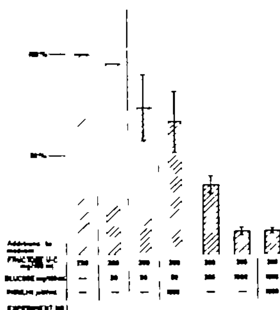


Fig. 2 Fructose metabolism of striated muscle. Rat diaphragm was incubated with ^{14}C fructose alone and with increasing concentrations of glucose with or without insulin. Fructose metabolism is quite considerable when glucose is absent. Increasing glucose concentrations and insulin inhibit the metabolism of fructose. (From ref. 4).

mg %, whereas free glucose appears to accumulate when insulin acts on higher glucose concentrations, thus inhibiting fructose phosphorylation at the hexokinase level (4, 15). If fructose is administered intravenously to rats together with anti-insulin serum, there is practically no incorporation of ^{14}C into diaphragm glycogen, indicating that fructose metabolism of muscle is probably very limited due to competitive inhibition by glucose (9).

FRUCTOSE AND GLUCOSE TRANSPORT INTO ADIPOSE TISSUE

In this respect, adipose tissue behaves in a completely different manner. Adipose tissue is the tissue of unlimited energy stores in the form of triglycerides. People with high caloric intake and low caloric output deposit their excess of calories almost exclusively in adipose tissue. On the other hand, adipose tissue fulfills the second most important goal of supplying the starving organism with free fatty acids from which up to 70 % of the energy is derived during fasting. Since adipose tissue does not have major energy requirements other than those needed for fat synthesis and fat mobilization, it can be looked upon as a sack of fat which needs the information of whether or not to take up glucose and to convert it to fat or to release free fatty acids from the triglyceride stores. In order to fulfill this task properly it needs to be practically impermeable to glucose in the basal state. This is, indeed, what one finds (2, 6). Adipose tissue *in vitro* will take up small amounts of glucose even in the absence of insulin, whereas adipose tissue *in vivo* does not appear to take up appreciable amounts of glucose if insulin is altogether lacking (9, 13). Glucose membrane transport is therefore the rate limiting step of glucose metabolism. This has been directly proven in very elegant experiments by Crofford and Renold (2). They incubated adipose tissue *in vitro* at 37 C with high concentrations of glucose and insulin and failed to detect any free glucose in adipose tissue cells. Only when the hexokinase activity was decreased by lowering the temperature to 17°C instead of 37 C there was actual accumulation of free glucose under the influence of insulin in the tissue. It would appear that the transport of glucose was relatively less affected by the low temperature than the hexokinase reaction so that the latter now became rate limiting. Under physiological circumstances, however, this does not seem ever to be the case, and transport of glucose is always rate limiting. We had postulated this many years ago on the basis of experiments carried out with ^{14}C-fructose with and without glucose and insulin (6, 10). It was found that glucose did

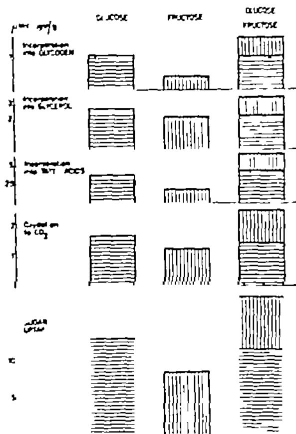


Fig 3 Additive metabolism of fructose and lucose by adipose tissue. Pooled epididymal rat tissue was incubated in the presence of ^{14}C-glucose (left), ^{14}C-fructose (middle), as well as with both sugars together (always in a concentration of 200 mg%) with ^{14}C-glucose or ^{14}C-fructose alternatively (right) The results of ^{14}C-fructose were added to those of ^{14}C glucose when both sugars were present in the medium to obtain total hexose metabolism (right column) The results show clearly that fructose metabolism is no inhibited by glucose and vice versa. (From ref. 6).

not inhibit ^{14}C-fructose uptake, oxidation and incorporation into total lipids of adipose tissue. Even in the presence of insulin, glucose and fructose were taken up by the tissue simultaneously and metabolized in an additive manner (6) (Fig 3) It can, therefore, be concluded that fructose is transported into adipose tissue by a different carrier than glucose and that glucose does not prevent fructose phosphorylation because it is not present in the free form in sufficient quantities to compete for phosphorylation at the hexokinase level.

Since transport is the limiting step of sugar metabolism by adipose tissue one can deduce useful information on the characteristics of the carrier mechanism by measuring total sugar metabolism by the tissue. Since about 70 % of the glucose or fructose taken up by adipose tissue is metabolized to CO_2 and total lipids, these two metabolic indices were taken to represent sugar uptake. Whereas glucose uptake of adipose tissue *in vitro* increases slowly when the glucose concentration in the medium is raised, fructose metabolism is very much dependent on the fructose concentration (4 13) The apparent K_m of glucose transport in the absence of insulin was estimated to be on the order of 4×10^{-4} M whereas the apparent K_m for fructose was approximately 4×10^{-2} M (4) Both sugars do not compete for the same carrier site on the membrane under basal conditions if no insulin is present. However fructose uptake is also stimulated, although to a much smaller extent than glucose uptake, by insulin. This insulin stimulation of fructose uptake is completely inhibited in the presence of glucose. It would, therefore appear that under basal conditions fructose is transported by an insulin independent carrier system but that the insulin effect on fructose uptake occurs by way of the glucose carrier (Fig 4)

THE ENZYMES OF FRUCTOSE METABOLISM IN ADIPOSE TISSUE

Fructokinase and fructose-1-phosphate aldolase are not present in adipose tissue (10) In all likelihood fructose is phosphorylated by hexokinase to fructose-6-phosphate and hence enters directly the glycolytic pathway Since the apparent lack of an enzyme in a homogenate does not prove that the respective enzyme is also absent in intact tissue, other evidence for or against direct phospho-

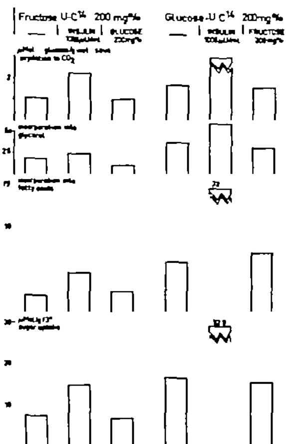


Fig. 4. Mutual independence of glucose and fructose metabolism of adipose tissue and stimulation by insulin. Pooled epididymal rat adipose tissue metabolizes almost as much fructose as glucose at hexose concentrations of 200 mg% in the medium. The effect of insulin on fructose uptake is small compared to glucose.

rylation of fructose to fructose-6-phosphate was searched for. Two approaches were used. Adipose tissue was incubated with fructose-1-^{14}C, glycogen isolated, hydrolyzed and C 1 recovered as ^{1} CO_2 after oxidation of glucose by bacteria. 98 / of ^{14}C in the glucose molecule was present in the C 1 position, indicating that the fructose molecule was not split to trioses and recondensed to fructose-1 6-diphosphate prior to incorporation into glycogen (10). The interpretation of these data has been rendered difficult by the failure to find fructose-1 6-diphosphatase activity in adipose tissue (17). If this latter enzyme were really missing, then one would pick up no ^{14}C from trioses since their recondensation is impossible. That would then mean that all fructose going to glycogen in adipose tissue must be incorporated after direct phosphorylation of fructose to fructose-6-phosphate. This can, indeed, be concluded from comparative studies on the incorporation of glucose-^{14}C and fructose-^{14}C, respectively into glycogen of adipose tissue. Although the relative amount of glucose and fructose taken up by adipose tissue which is incorporated in glycogen may not be the same for both sugars, one does, nevertheless, find a certain relationship between sugar uptake and glycogen synthesis for both sugars. Therefore, fructose appears to be directly phosphorylated to fructose-6-phosphate and to share the same fate as glucose which enters glycolysis as glucose-6-phosphate. Insulin enhances glycogen formation from ^{14}C-glucose to a relatively greater extent than from ^{14}C-fructose (7). Since fructose and glucose use different carriers to enter the adipocyte, these metabolic differences may be due to the compartmentalization of the adipocyte.

THE METABOLIC FATE OF FRUCTOSE IN ADIPOSE TISSUE

Transport into the cell rather than enzyme activity determines fructose and glucose metabolism of adipose tissue to a major extent. However fructose like glucose metabolism is modulated to some degree by the activity of the triglyceride lipase. When triglyceride lipase is stimulated, free fatty acids are produced and α-glycerophosphate originating from the hexose will be used for the partial reesterification of free fatty acids. Therefore, lipolysis stimulates the incorporation of ^{14}C-fructose into glyceride-glycerol of total lipids. Lipolysis is stimulated by insulin-antagonistic hormones such as glucagon,

Table I. *Glucose and fructose transport characteristics in adipose tissue of normal, acutely and subacutely diabetic rats*

Pooled epididymal adipose tissue was incubated with 3 different concentrations of either ^{14}C-glucose or ^{14}C fructose (50 mg%, 200 mg% and 800 mg%). The results of $^{14}CO_2$ and total lipids were added together and taken as a measure of hexose transport. (From ref. 4)

Rats	Glucose Transports into Adipose Tissue K_m moles/liter	Glucose Transports into Adipose Tissue V_{max} μmoles/g/hr	Fructose Transports into Adipose Tissue K_m moles/liter	Fructose Transports into Adipose Tissue V_{max} μmoles/g/hr
Normal fed	4.7×10^{-3}	4.5	4.1×10^{-2}	11.3
Acutely alloxan-diabetic, insulin withdrawal	3.0×10^{-3}	2.5	3.0×10^{-2}	7.2
Subacutely alloxan-diabetic, no insulin	4.0×10^{-3}	1.5	9.3×10^{-2}	1.5

adrenaline, ACTH and growth hormone. All these hormones increase the uptake of glucose by adipose tissue to some extent (1 14). Relatively *more glucose is oxidized* in the tricarboxylic acid cycle whereas the pentose phosphate shunt is not activated by these hormones. No similar experiments have been carried out with fructose. Since the uptake of fructose rather than enzyme activities determine how much fructose is being metabolized, it is likely that the lipolytic hormones have similar effects on fructose as on glucose metabolism.

Thus, overall fructose metabolism depends in the first place on the fructose concentration. Insulin in the presence of cold glucose leads to a relatively increased incorporation of ^{14}C *of fructose into fatty acids, whereas* lipolytic hormones stimulate incorporation into glyceride-glycerol (7)

FRUCTOSE METABOLISM OF ADIPOSE TISSUE FROM DIABETIC ANIMALS

Fructose and glucose metabolism of adipose tissue of acutely and chronically diabetic rats have been compared in a rather extensive study (4). Glucose and fructose uptake decreased in a parallel manner 48—72 hours after insulin withdrawal in alloxan-diabetic rats. Hexose uptake is still normal when expressed on a weight basis, but is clearly smaller when expressed per fat pad or per rat. At this early stage of insulin deficiency *the response to insulin in vitro is unimpaired.* At a later stage (120—144 hours after alloxan administration) fructose and glucose uptake of adipose tissue are very much decreased and so is the insulin response of the tissue. This is demonstrated by the results in Fig 5. In diabetic adipose tissue membrane transport remains rate limiting for glucose and fructose metabolism. The apparent K_m of glucose and fructose transport does not change whereas there is a 3-fold decrease of the apparent V_{max} of glucose transport and a 7 fold decrease of the V_{max} of fructose transport (4) (Table I). Interestingly the two different carrier mechanisms appear to be altered in a similar manner by insulin deficiency. These findings indicate that *insulin deficiency leads to a loss of both* glucose and fructose carrier. This is in keeping with the results of very interesting experiments which suggested that insulin may stimulate the formation of glucose carrier in adipose tissue membranes (12). The carrier system which is left in diabetic adipose tissue appears to have similar affinities for fructose and glucose as that of normal tissue. Insulin lack seems to reduce the quantity of the carrier rather than change its quality

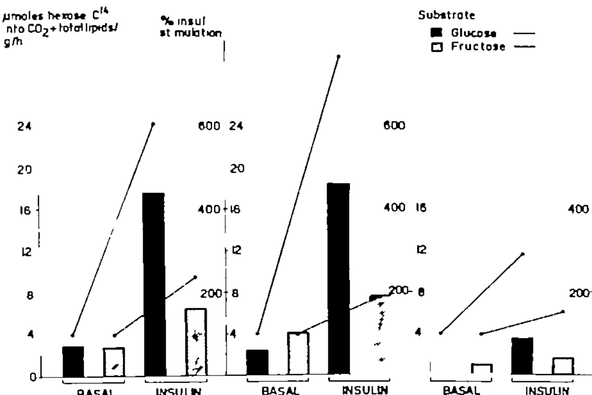


Fig 5 Fructose and glucose metabolism by pooled epididymal adipose tissue from normal (left) acutely diabetic (middle) and chronically diabetic rats (right) Glucose and fructose metabolism and their response to insulin expressed per g of tissue are not reduced in rats which have been deficient in insulin fo 48—72 hours (middle) Basal uptake and insulin sensitivity are markedly reduced after 120—144 hours of insulin lack (right) (From ref. 4)

The metabolic fate of glucose and fructose is similarly affected in diabetic adipose tissue (Fig. 6) The percentage of hexose taken up by adipose tissue which is incorporated into fatty acids increases in a parallel manner with the hexose uptake, whereas incorporation into glyceride-glycerol decreases conversely Since hexose uptake of diabetic adipose tissue is drastically reduced, fatty acid synthesis is minimal. A high percentage of the ^{14}C of hexose is incorporated into glyceride-glycerol. Whereas up to 70 % of hexose carbon-14 is incorporated into fatty acids by adipose tissue of normal rats at high rates of hexose uptake, diabetic tissue incorporates less than 20 % into fatty acids at maximal rates of hexose uptake. The decreased hexose transport appears to be primarily responsible for the impaired fatty acid synthesis by diabetic adipose tissue.

FRUCTOSE METABOLISM OF ADIPOSE TISSUE IN VIVO

The investigation of the direct utilization of fructose by adipose tissue in vivo is rendered difficult by the rapid conversion of fructose to glucose. Thus, when one tries to trace the fate of carbon 14 from injected ^{14}C-fructose one cannot distinguish between direct incorporation of ^{14}C fructose on one hand and the incorporation of ^{14}C-glucose deriving from labelled fructose on the other hand. From our *in vitro* results we dare to extrapolate to the *in vivo* situation and are pretty certain that insulin *in vivo* will not stimulate

Table I. *Glucose and fructose transport characteristics in adipose tissue of normal, acutely and subacutely diabetic rats*

Pooled epididymal adipose tissue was incubated with 3 different concentrations of either ^{14}C-glucose or ^{14}C-fructose (50 mg%, 200 mg% and 800 mg%). The results of $^{14}CO_2$ and total lipids were added together and taken as a measure of hexose transport. (From ref. 4)

Rats	Glucose Transport into Adipose Tissue		Fructose Transport into Adipose Tissue	
	K_m moles/liter	V_{max} μmoles/g/hr	K_m moles/liter	V_{max} μmoles/g/hr
Normal fed	4.7×10^{-3}	4.5	4.1×10^{-3}	11.8
Acutely alloxan-diabetic, insulin withdrawal	3.0×10^{-3}	2.5	3.0×10^{-3}	7.2
Subacutely alloxan-diabetic, no insulin	4.0×10^{-3}	1.5	9.3×10^{-3}	1.8

adrenaline, ACTH and growth hormone. All these hormones increase the uptake of glucose by adipose tissue to some extent (1, 14). Relatively more glucose is oxidized in the tricarboxylic acid cycle whereas the pentose phosphate shunt is not activated by these hormones. No similar experiments have been carried out with fructose. Since the uptake of fructose rather than enzyme activities determine how much fructose is being metabolized, it is likely that the lipolytic hormones have similar effects on fructose as on glucose metabolism.

Thus, overall fructose metabolism depends in the first place on the fructose concentration. Insulin in the presence of cold glucose leads to a relatively increased incorporation of ^{14}C of fructose into fatty acids, whereas lipolytic hormones stimulate incorporation into glyceride-glycerol (7).

FRUCTOSE METABOLISM OF ADIPOSE TISSUE FROM DIABETIC ANIMALS

Fructose and glucose metabolism of adipose tissue of acutely and chronically diabetic rats have been compared in a rather extensive study (4). Glucose and fructose uptake decreased in a parallel manner 48—72 hours after insulin withdrawal in alloxan-diabetic rats. Hexose uptake is still normal when expressed on a weight basis, but is clearly smaller when expressed per fat pad or per rat. At this early stage of insulin deficiency the response to insulin *in vitro* is unimpaired. At a later stage (120—144 hours after alloxan administration) fructose and glucose uptake of adipose tissue are very much decreased and so is the insulin response of the tissue. This is demonstrated by the results in Fig. 5. In diabetic adipose tissue membrane transport remains rate limiting for glucose and fructose metabolism. The apparent K_m of glucose and fructose transport does not change whereas there is a 3-fold decrease of the apparent V_{max} of glucose transport and a 7 fold decrease of the V_{max} of fructose transport (4) (Table I). Interestingly the two different carrier mechanisms appear to be altered in a similar manner by insulin deficiency. These findings indicate that insulin deficiency leads to a loss of both glucose and fructose carrier. This is in keeping with the results of very interesting experiments which suggested that insulin may stimulate the formation of glucose carrier in adipose tissue membranes (12). The carrier system which is left in diabetic adipose tissue appears to have similar affinities for fructose and glucose as that of normal tissue. Insulin lack seems to reduce the quantity of the carrier rather than change its quality.

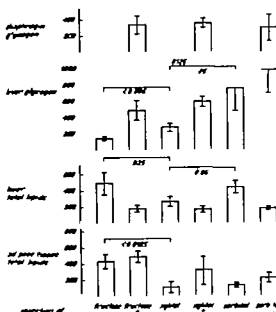


Fig. 7 Fructose metabolism by dipose tissue of streptozotocin-diabetic rats in vivo. The metabolism of [14]C fructose was compared with that of [14]C xylitol and [14]C-sorbitol. These two polyols are not directly taken up by adipose tissue but rather after conversion to [14]C-glucose. The greater [14]C incorporation into total lipids seen after [14]C fructose administration indicates that fructose is taken up as such by adipose tissue in vivo (From ref 9)

want to make here is the fact that fructose is being utilized by adipose tissue *in vivo* independently of insulin, whereas xylitol and sorbitol are utilized only after hepatic con version to glucose and under insulin stimula tion.

CONCLUSIONS

Adipose tissue takes up fructose in a concentration dependent fashion. At a concentration of 100 mg /s, fructose and glucose uptake by adipose tissue are equal. Fructose transport is stimulated by insulin only in the absence of glucose. Fructose appears to be transported by a carrier which is distinctly different from the glucose carrier

The metabolism of fructose by adipose tissue depends mainly on the rate of entry into the cell which in turn depends on the concentration in the medium. Adipose tissue *in vivo* plays a minor role in the removal of glucose from the blood even in the presence of insulin. The role of adipose tissue consists in the unlimited storage of carbohydrate and fat in the form of trigly cerides on one hand and in the provision of free fatty acids as a source of energy replacing glucose

Adipose tissue is not very important in the overall metabolism of either glucose or fructose *in vivo* However fructose leads to increased reesterification of free fatty acids, so that their level in the blood decreases which in turn leads to increased glucose oxidation. In contrast to glucose, insulin is not necessary for this effect of fructose.

Adipose tissue of diabetic animals shows a decrease of glucose as well as of fructose uptake *in vitro* and a relative loss of insulin sensitivity *In vivo* considerable incorpora tion of [14]C-fructose takes place in adipose tissue of streptozotocin-diabetic rats. Fruc tose decreases lipolysis more markedly than insulin alone by providing more α-glycerophosphate for reesterification.

Fructose has proven to be an extremely interesting sugar with regard to the elucida tion of regulatory processes in adipose tissue the most important of which are sugar transport on one hand and the lipolytic activity on the other hand.

REFERENCES

1. Cahill, G. F J Leboeuf, B. & Flinn, R. B. Studies on rat adipose tissue in vitro. VI. Effect of epinephrine on glucose metabolism. J biol. Chem. 235 1246, 1960
2. Crofford, O. B. & Renold, A. E. Glucose uptake by incubated epididymal adipose tissue. Rate limiting steps and site of insulin action. J biol. Chem. 240 14, 1965.
3. Di Pietro, D L. Hexokinase of white adipose tissue. Biochim. biophys. Acta 67 305, 1963.

4. Froesch, E. R. Fructose metabolism in adipose tissue from normal and diabetic rats, in Hand book of Physiology section on Adipose Tissue, (eds. A. E. Renold and G F Cahill, Jr.), pp. 281—293, American Physiological Society Washington D C., 1965
5. Froesch, E. R. Essential fructosuria and hereditary fructose intolerance, in The Metabolic Basis of Inherited Disease, 3rd ed. (eds. J B. Stanbury J B. Wyngaarden and D S. Fredrickson), pp. 131—148, Mac Graw Hill, New York, 1972.
6. Froesch, E. R. & Ginsberg, J L. Fructose metabolism of adipose tissue. I. Comparison of fructose and glucose metabolism in epididymal adipose tissue of normal rats. J biol. Chem. 237 3317 1962.
7 Froesch, E. R., Waldvogel, M., Meyer U A., Jakob A. & Labhart, A. Effects of 5-methyl pyrazole-3-carboxylic acid on adipose tissue. I. Inhibition of lipolysis, effects on glucose, fructose and glycogen metabolism in vitro and comparison with insulin. Mol. Pharmacol. 3 429 1967
8. Froesch, E. R., Wolf, H. P Baitsch, H., Prader A. & Labhart, A. Hereditary fructose intolerance an inborn defect of hepatic fructose-1 phosphate splitting aldolase. Amer J Med. 34 151 1963.
9. Froesch, E. R., Zapf, J Keller U & Oelz, O Comparative study of the metabolism of U-[14]C-fructose, U-[14]C-sorbitol and U-[14]-C xylitol in the normal and streptozotocin diabetic rat. Europ. J clin. Invest. 2 8, 1971.
10 Ginsberg, J L. Fructosestoffwechsel des Fettgewebes. II. Abbauwege der Fructose im epididymalen Fettgewebe der Ratte. Zschr. ges. exp. Med. 139 101, 1965.
11 Keller U & Froesch, E. R. Metabolism and oxidation of U-[14]C-glucose, xylitol, fructose and sorbitol in the fasted and in the streptozotocin-diabetic rat. Diabetologia 7 349 1971.
12. Kono, T Destruction and restoration of the insulin effector system of isolated fat cells. J biol. Chem. 244 5777 1969
13. Leonards, J R. & Landau, B. R., Metabolism of fructose by adipose tissue and the effect of insulin. Endocrinology 74 142, 1964
14. Lynn, W S., Mac Leod, R. M. & Brown, R. H Effects of epinephrine, insulin and corticotrophin on the metabolism of rat adipose tissue J biol. Chem. 235 1904, 1959
15. Morgan, H. E., Henderson, M. J Regen, D. M & Park, C. R. Regulation of glucose uptake in muscle. I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated perfused heart of normal rats J biol. Chem. 236 253, 1961
16. Steinitz, H., Steinitz, K. & Misrachi, O Essentielle Fructosurie Untersuchungen des intermediären Stoffwechsels bei intravenöser Fructosebelastung. Schweiz. med. Wschr 93 56, 1963.
17 Weber G Banerjee, G & Ashmore, J Activities of enzymes involved in glycolysis, gluconeogenesis and hexose monophosphate shunt in rat adipose tissue. Biochem. biophys Res. Comm. 3 182, 1960

Acta med. scand. Suppl. 542, pp. 47—56

CONTROL OF HEPATIC FRUCTOSE-METABOLIZING ENZYMES FRUCTOKINASE, ALDOLASE AND TRIOKINASE

Richard C. Adelman

From Fels Research Institute and Department of Biochemistry Temple University School of Medicine, Philadelphia, Pennsylvania 19140 U.S.A.

Abstract. Physiological factors which may regulate the catalytic activity of three key enzymes of hepatic fructose metabolism, fructokinase, aldolase and triokinase, are reviewed. Possible relationships between these potential control mechanisms and hepatic fructose metabolism per se are discussed t three distinct levels 1) characterization in vitro of purified enzyme preparations 2) variation of enzyme activity in vivo under various dietary and hormonal conditions and 3) variation of enzyme activity in vivo following development of liver tumors of different growth rate and degree of cellular differentiation.

The ability of an organism to adapt to environmental change is the basis for the maintenance of a dynamic state in normally functioning cells. Such adaptation requires tight control and regulation of specific enzyme-catalyzed reaction rates. Whereas the focus of past biochemical research was on delineation of broad pathways, in recent years emphasis has shifted towards regulatory mechanisms of specific metabolic pathways and their constituent enzyme-catalyzed reactions. It is the purpose of this article to review certain aspects of the metabolism of fructose in such a light.

A host of experiments *in vivo* and *in vitro* has established the liver as the most important organ of fructose metabolism, accounting for up to 85 % of the orally administered sugar (for example, see 23 32, 38, 45 67 74) The main results of earlier investigations concerning the metabolism of fructose in liver indicate that it is not introduced as a hexose into the main stream of general carbohydrate metabolism, but only after cleavage into 3-carbon units (45 46 51). Furthermore, there is a small number of acknowledged enzymes which have a specific significance for hepatic fructose metabolism fructokinase, which phosphorylates fructose in the 1-position an aldolase which cleaves fructose-1-phosphate (F 1 P) to dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde (GA) and perhaps triokinase, which phosphorylates GA, forming glyceraldehyde-3-phosphate (GAP) Where applicable this review will deal with control of these hepatic enzymes at the following levels of organization 1) characterization *in vitro* of purified preparations 2) susceptibility *in vivo* to nutritional

and hormonal manipulation and 3) extent of altered genetic expression in hepatic tumors of varying growth rate and degree of cellular differentiation.

CHARACTERIZATION OF HEPATIC FRUCTOSE-METABOLIZING ENZYMES

Fructokinase Liver fructokinase was first examined by Leuthardt and Testa and by Kuyper both of whom demonstrated its broad specificity showing that it phosphorylates L-sorbose, D-tagatose, L-arabinose, L-galactoheptulose and D-altroheptulose, as well as D-fructose to form the corresponding 1 phosphate (cf. ref. 26)

Fructokinase activity in the liver is substantially higher that of hexokinase (68) and the former enzyme has a great affinity toward fructose according to Hers (24) the Michaelis constant is about 0.5 mM. Although fructose is readily phosphorylated in liver by hexokinase to fructose-6-phosphate (F-6-P) its low activity and its K_m for fructose of 2 to 6 mM (15 24) as compared with a K_m for glucose of 0 1 mM (68) makes it unlikely that F-6-P is formed in appreciable quantities under normal circumstances.

F 1 P was inducated as the reaction product in liver by several investigators (12, 33 38) and finally was verified by Leuthardt and Testa (cf ref 26) who obtained the ester in analytically pure from by means of a purified enzyme According to Hers (26) an ester with the properties of F 1 P is formed also when muscle extracts are incubated with large quantities of fructose.

Although the hexokinases of yeast, brain and muscle are able to react with fructose as well as with a whole series of additional sugars to produce the corresponding 6-phosphate these enzymes possess a very much lower affinity for fructose than for glucose (11 61). Thus the reaction with fructose is strongly inhibited by glucose. In consideration of the great importance of muscle carbohydrate metabolism, the behavior of the muscle enzyme toward fructose is of great interest. According to Colowick (11), at a concentration of 0.28 mM the enzyme is 90 % saturated with glucose, but only 5 % with fructose. This explains why the conversion *of fructose in muscle is very slight* under physiological conditions. The need of a specific fructokinase that is not inhibited by glucose was made comprehensible from this condition (13) If the liver contained only one hexokinase of the type of the yeast, muscle or brain enzyme, because of the high glucose concentration, there must be little if any utilization of fructose

Requirements by fructokinase for K^+ and Mg^{++} were demonstrated by several workers (25 42, 67), and it was shown that the Mg^{++} concentration must equal or exceed the concentration of ATP in order to attain maximal activity In fact, excess ATP was shown to be so strongly inhibitory that the reaction velocity was reduced to zero when the ATP to-Mg^{++} ratio reached a value of five.

Much greater fructose phosphorylation was demonstrated under aerobic than under anaerobic conditions (67 60) After Slein (59) showed that the decreased fructose consumption by rat liver homogenates under anaerobic conditions was not due to limiting ATP Parks et al. (42) explored the possibility of product inhibition. They ruled out F 1 P as the inhibitor because addition of fluoride, while resulting in a large accumulation of F 1 P did not inhibit fructose utilization (12, 60)

By adding creatine phosphate and purified muscle ATP creatine phosphotransferase (31) in nonlimiting amounts to the liver fructokinase assay system, ADP could be converted to ATP as rapidly as it was formed. By thus preventing the accumulation of ADP they not only enhanced fructokinase activity but also achieved a proportionality between the amount of enzyme and fructose disappearance, thus providing a useful assay for all

in purification (42) They concluded that the effect of aerobic conditions on fructokinase activity was due to removal of ADP presumably by oxidative phosphorylation to ATP Although fructokinase had been purified only 10 to 20-fold previously (37) using this assay method Parks et al. (42) developed a reliable procedure for purifying beef liver fructokinase 150-fold.

The striking inhibition of fructokinase activity by ADP and excess ATP could well play an important role in the regulation of hepatic fructose metabolism. Although inhibition by excess of substrate is not rare the unique aspect of this particular case is that in the Michaelis constant region slight variation of ATP or Mg^{++} concentrations may have relatively great effects on the reaction velocity The obvious conclusion is that cellular enzyme control may normally be exerted by alteration of adenine nucleotide or Mg^{++} concentrations associated with various physiological processes.

Recently new spectrophotometric and radiochemical assay procedures for measuring fructokinase activity were reported (1) This enzyme subsequently was purified from the liver of adult rats by acid and heat treatment, ammonium sulfate fractionation, and chromatography on Sephadex G-200 and diethyl aminoethyl cellulose. The enzyme preparation has specific activity of 15 to 30 units per mg of protein at 25 and was purified approximately 1 000-fold from the 100 000 × g supernatant. Purified liver fructokinase has a K_m of 0.2 to 0.5 mM for fructose and 1 to 2 mM for magnesium adenosine triphosphate, and the enzyme reaction produces equimolar amounts of F 1 P and ADP Enzyme phosphorylates D-fructose with ATP 2-dATP or 3-dATP and also phosphorylates L-sorbose, D-xylulose, and L-galactoheptulose with ATP It is stimulated by cysteine and other thiol, inhibited by p-chloromercuribenzoate and has an activation energy of about 11 kcal per mole and a Q_{10} of 2.

Similar purification and characterization of this enzyme, including more extensive studies on the role of K^+ were reported by Sanchez et al. (52, 53)

Aldolase In liver F 1 P is immediately cleaved into DHAP and D-GA (27) It is now well established that the predominant, if not the only aldolase of liver differs from the muscle aldolase in composition (50) and in its physical, kinetic, and immunochemical properties (6, 14, 16, 43, 50 54 63) Although it had been claimed that the aldolase activities of liver toward fructose 1 6-diphosphate (FDP) and F 1 P were due, respectively to different species of enzyme (30) there seems little doubt from studies of several investigators that a single enzyme species can account for activities toward both substrates. If the muscle type aldolase is present in rabbit liver it must be less than 1 % of the total as determined immunochemically (48) Several of those differences described above, as well as others, recently were confirmed using homogeneous preparations of the liver enzyme (9 20, 21). These results leave hardly any doubt that liver contains only a single species of aldolase that is responsible for the activity toward both substrates.

Thorough kinetic studies of liver and muscle aldolase were recently completed by Spolter et al. (63), who demonstrated that liver aldolase differs from both native and carboxypeptidase-treated muscle aldolase at the binding site of the substrate to the enzyme. Among several inhibitors of both liver and muscle aldolase that were discovered, the most significant were the adenine nucleotides. Muscle aldolase was competitively inhibited by ATP > ADP > AMP Moreover the ATP inhibition was reversed by Mg^{++} On the other hand, liver aldolase was competively inhibited by AMP > ADP but not at all by ATP It is not inconceivable that these inhibitory actions play a physiological role in controlling the activity of aldolase in *vivo* As a further

result of these kinetic studies, the Michaelis constant for FDP and F 1 P of liver aldolase was demonstrated to be 0.004 mM and 2 mM, respectively and of muscle aldolase 0.014 mM and 7.0 mM, respectively

Triokinase. The further fate of D-GA formed by aldolase in liver is not yet firmly established. Three possible sequences may be envisioned 1) reduction to glycerol and subsequent phosphorylation to α-glycerophosphate 2) oxidation to glyceric acid with its subsequent phosphorylation and 3) direct phosphorylation to GAP Although it is possible that under appropriate conditions each of the three above-mentioned routes can be used, the existence of a specific triokinase in liver capable of directly phosphorylating D-GA to GAP implies that this step may be the chief route by which D-GA formed from fructose is converted to glucose. Of the potential GA-utilizing enzymes in liver only triokinase will be considered in this article.

According to Hers and Kusaka (26 27) there does exist in the liver a kinase which is able to phosphorylate D-GA directly By ammonium sulfate fractionation these authors obtained from guinea pig liver a protein fraction which produced triose phosphate on F 1 P on incubation with D-GA. The phosphorylation of GA was not competitively inhibited by glycerol in this mixture. These observations established the existence in liver of a kinase specific for GA, denoted as triokinase.

Lindberg (37) first demonstrated that GA can be phosphorylated to GAP in dialyzed kidney extracts. Hers and Kusaka (27) suggested that in crude extracts, rich in triose phosphate isomerase and aldolase, the GAP thus formed would isomerize to DHAP and then react with a second molecule of GA to form F 1 P Triokinase also was partially purified by ammonium sulfate treatment, and was shown to phosphorylate at approximately equal rates, D-GA, D,L-GA and dihydroxyacetone.

Heinz and Lamprecht (22) reported the preparation of triokinase from beef liver in about 50-fold enrichment. They proposed that triokinase phosphorylated the primary hydroxyl group of D-GA and dihydroxyacetone, yielding the corresponding triose phosphate and ADP L-GA, D-glycerate, glucose and fructose were not found to be substrates. The Michaelis constant for D-GA was 0 125 mM, for dihydroxyacetone 0 02 mM and for D,L-GA 0.33 mM. Optimal triokinase activity was demonstrated at pH 7 0

Hers (28) reported the purification of triokinase (free of glycerokinase) from guinea pig liver and subsequently discovered that ITP cannot replace ATP as a phosphate donor and that optimal activity occurred between pH 7.2 and 7.8

Although addition of isobutyramide to impure reaction mixtures, to inhibit NADH-dependent reduction of GA by alcohol dehydrogenase facilitated development of a simple, spectrophotometric assay procedure (2) attempts to purify and to characterize this enzyme have been largely unsuccessful

NUTRITIONAL AND HORMONAL REGULATION

Although rates of fructose utilization have been determined in liver slices from fasted (78) adrenalectomized (4) and alloxan-diabetic (10 40) rats, relatively little information is available concerning dietary and hormonal effects on the enzymes involved in hepatic fructose metabolism, other than those described by Adelman et al. (3). Marked changes were observed in total liver activity of fructokinase, aldolase and triokinase when rats were maintained under various dietary and hormonal conditions. In contrast specific activities in terms of liver weight did not change greatly

All three activities decreased to 50 or less of their normal total activity on fasting

for 48 to 72 hours, and were restored completely in 24 hours by fructose feeding With glucose feeding, recovery was complete for aldolase but only partial for fructokinase and triokinase. No recovery of activity was detectable following administration of a high protein diet to fasted rats. Long term feed ing of fructose resulted in the maintenance of a considerably higher level of all three enzymes over that on a high fat or high protein diet.

These enzyme activities were unchanged in alloxan-diabetic rats. Fructokinase activity of fed adrenalectomized rats was at the fasting level of normal rats and was neither lowered further on fasting nor increased by subsequent feeding of fructose or glucose. In contrast, aldolase and triokinase activities were normal and decreased sharply on fast ing, but did not recover on subsequent glucose or fructose feeding Essentially the same pattern was found for hypophysectomized as for adrenalectomized rats.

The action of fructose in the recovery of fructokinase and triokinase activity after fasting provides additional examples of substrate induction of phosphotransferases, anal ogous to that of glucose on hepatic glucokinase (56). However the action of fructose on triokinase probably is indirect, by virtue of its metabolic conversion to D-GA (36) The induction of aldolase activity by both glucose and fructose was not unexpected, inasmuch as this enzyme is also required for glucose catabolism. However the decrease in this enzyme activity on fasting was sur prising since substrate induction in the opposite direction might have been anticipated in consequence of the increased gluconeogenesis that occurs in this condition. The lack of responsiveness to dietary protein distinguishes these adaptations from those resulting from general protein repletion (39), or those due to induction of specific enzymes of amino acid metabolism (34). Although the long and short term feeding experiments suggest that fructose acts as an enzyme inducer similar effects are displayed also by glucose, and the activity fluctuations accompany changes in liver weight. Also, fructose feeding apparently can increase the activities of hepatic enzymes not associated directly with fructose metabolism and has no effect on others (18 19). These observations argue against a highly specific substrate induction. On the other hand, the rise in liver weight on fructose feeding is not due to a general increase of protein, but rather to a large increase of liver glycogen.

The absence of any effect of alloxan diabetes on the activities of all three enzymes implies that they are not affected by lack of insulin. This is in accord with previous observations that the utilization of fructose and its conversions to glucose, CO_2, and glycogen are unimpaired in liver slices of diabetic rats (10 40 47). It is evident that the adrenal glands are required for adaptation to dietary fructose and that these responses may be mediated via glucocortical steroids. The lack of responsiveness to fructose feeding as well as the low levels of activity of these enzymes in hypophysectomized rats may also be attributable to lack of glucocorticoid secretion, since these deficiencies were largely corrected by hydrocortisone administration.

Despite the marked fluctuations in activity described briefly above the enzyme levels were still sufficiently high to account for known rates of fructose metabolism in intact cells. According to Spiro and Hastings (62) liver slices incubated aerobically at 37 util ize fructose at a rate of 240 μmoles per g of tissue per 90 min, corresponding to 2.7 μmoles per g of tissue per min. This value is also in good agreement with previous values based on total fructose disappearance at 30 in adult rat liver (68) fructose disappearance in liver homogenates at 37 (67) and conversion to glucose in liver slices at 37 (7). These values are strikingly close to the assay value of between 2.1 and 2.5 μmoles per g of tissue per min for fructokinase measured at 25 (3).

The same conclusion applies to aldolase activities, which are somewhat higher than those of fructokinase Although triokinase activity was the lowest of all three enzymes, the rate of 1 μmole per min per g of tissue at 25 again appears to be sufficient to account for the known rates of conversion of fructose to glucose.

ENZYME LEVELS IN HEPATIC TUMORS

Several biochemical studies of a series of slow-growing well-differentiated rat liver tumors, induced by feeding chemical carcinogens, have revealed that the neoplastic transformation may occur without the obligatory loss of enzyme associated with normal hepatic function. However no clear pattern of retention or deletion has emerged. Some enzymes are retained, some are lost, some are retained but regulatory control may be reduced or deleted, and in some instances typical hepatic enzymes appear to be replaced by non hepatic or fetal types (for example 41 65 73)

An especially striking feature revealed in earlier studies of rapidly growing liver tumors is the loss of certain enzymes characteristic of hepatic carbohydrate metabolism, such as fructose-1 6-diphosphatase (71) and glucose-6-phosphatase (72) As a result of such metabolic lesions it was proposed that the metabolic pattern of these hepatomas approaches that of the muscle with a capacity to produce large amounts of lactate (6, 69) Subsequent reports of related changes in hepatic carbohydrate metabolism include replacement of glucokinase the predominant glucose ATP phosphotransferases of normal liver by hexokinase(s) in poorly differentiated hepatic tumors (57 75) and also an apparent tendency toward increase in the molecular species characteristic of non hepatic or fetal liver aldolase with increasing growth rate of liver tumors (49 55 64).

As discussed earlier there is a clear-cut difference between the metabolic utilization of fructose in liver and in muscle (27). In the liver fructose is metabolized by fructokinase and aldolase and the molecule enters into glycolysis or glycogenesis at the triose phosphate stage In muscle fructose is phosphorylated by hexokinase and it undergoes subsequent reactions as F-6-P As a result of this difference, and because several properties of carbohydrate metabolism in some liver tumors tend to approach the metabolic pattern of muscle, it became of interest to assay the behavior of fructose metabolizing enzymes in liver tumors of varying growth rate.

With only a few exceptions, all three enzymes of fructose metabolism were present in well-differentiated liver tumors (2 17) Fructokinase and triokinase were much lower in activity than in liver but aldolase, though somewhat variable, was usually in the range of liver activity and the low FDP/F 1 P activity ratios indicated that the aldolase was nearly exclusively of the liver type. These findings represent only a small portion of a growing body of evidence that many enzymes associated with liver-specific functional activities are retained in these chemically induced, transplantable tumors, not only through the initial neoplastic transformation, but through many transplant generations (41). In contrast, the poorly differentiated tumors were devoid of fructokinase and triokinase, and the aldolase activity though high, was predominantly or entirely of the non-hepatic type More recently aldolases were purified to homogeneity from rat liver muscle and the Novikoff or Yoshida hepatoma (both characteristic of poorly differentiated liver tumors) (9 20 29) Rigorous characterization in physical and chemical terms provided support for the conclusion that liver aldolase is replaced by muscle aldolase during the development of these liver tumors.

The enzyme data are in good accord with previous results on fructose metabolism

Sweeney et al. (65) studied the metabolism of fructose-U-^{14}C in slices of some of these tumors and found that net fructose uptake was lower in tumors than in liver· its conversion to glucose also was very low and was decreased further in tumors with high growth rate. However conversion of the labeled carbon to CO_2, lactate and glycogen was actually higher in the fast growing, poorly-differentiated tumors. Although at first glance these findings appear to be incompatible with the lack of fructokinase in the poorly-differentiated tumors, these tumors do have high hexokinase activity (58) and fructose, in the concentrations used by these investigators, namely 30 mM, is a good substrate for this enzyme. The low rate of conversion of fructose to glucose can be attributed to low or negligible levels of fructose-1,6-diphosphatase and glucose-6-phosphatase in these tumors (70) Experiments of Ashmore et al. (5) with fructose-1 ^{14}C provided further evidence for utilization of fructose via fructokinase in well differentiated tumors and via hexokinase in poorly-differentiated tumors. They demonstrated that glycogen synthesis from fructose in slices of poorly-differentiated liver tumors proceeded without appreciable cleavage of hexose, therefore via hexokinase, whereas in the well-differentiated 7800 and 5123D tumors it occurred via intermediary triose formation, thus presumably via fructokinase.

From previous work on these three enzymes in liver (see above and 61) it appears that they respond to dietary changes as a unit, suggesting that their control may be coordinated. Weber (70) had proposed that hepatic enzymes involved in sequential metabolic steps may be regulated as a single unit. Despite a few glaring exceptions, it is clear that in the well-differentiated tum rs all three enzymes are usually present, whereas, in the poorly-differentiated ones all are absent or nearly so. Within this pattern, however there are such wide variations in activity that one must assume such coordination does not extend to the quantitative expression of enzyme activities.

ACKNOWLEDGEMENTS

Preparation f this manuscript was supported by research grants HD-05874, HD-04382 and CA 05222 from the N tional Institutes of Health, and P-502 from the American Cancer Society

REFERENCES

1. Adelman, R. C., Ballard, F J & Weinhouse, S.. Purification and properties of rat liver fructokinase. J biol. Chem. 242 3360, 1967
2. Adelman, R. C., Morris, H. P & Weinhouse S. Fructokinase, triokinase, and aldolases in liver tumors of the rat. Cancer Res. 27 2408, 1967
3. Adelman, R. C., Spolter P D & Weinhouse, S. Dietary and hormonal regulation of enzymes of fructose metabolism in rat liver J biol. Chem. 241 5467 1966.
4. Ashmore, J Cahill, G F J Hillman, R. & Renold, A. E. Adrenal cortical regulation of hepatic glucose metabolism. Endocrinology 62. 621, 1958.
5. Ashmore, J Sweeney M. J Morris, H. P & W bar G. Change from liver type to muscle type fructose metabolism in hepatomas. Biochim. biophys. Acta 71 451 1963.
6. Ashmore, J Weber G. & Landau, B. R. Isotope studies on the pathways of glucose-6-phosphate metabolism in the Novikoff hepatoma. Cancer Res. 18 974, 1958.
7. Ballard, F J Pathways of fructose conversion into glucose in foetal rat liver. Biochem. J 97 365, 1965.
8. Blostein, R. & Rutter W J.. Comparative studies of liver and muscle aldolase. J biol. Chem. 238 3280, 1963.
9. Brux, L. W Lacko, A. G Gracy R. W Adelman, R. C. & Horecker B. L. Muscle-type aldolase isolated from liver tumor Biochem. biophys. Res. Commun. 36 994, 1969.
10. Chernick, S. S. & Chaikoff, I. L. Two blocks in carbohydrate utilization in the liver of the diabetic rat. J biol. Chem. 188 389, 1951.
11. Colowick, S. P Transphosphorylating enzymes of fermentation. The Enzymes (eds., J B. Sumner and K. Myrbäck), II, 130, Academic Press, Inc., New York, 1951.

12. Cori, C. F. Phosphorylation of glycogen and glucose. Biol. Sympos. 5 131, 1941
13. Cori, G. T., Ochoa, S., Slein, M. W. & Cori, C. F. The metabolism of fructose in liver. Isolation of fructose-1 phosphate and inorganic pyrophosphate. Biochim. biophys. Acta 7 304, 1951.
14. Dahlqvist, A. & Crane, R. K. The influence of the method of assay on the apparent specificity of rabbit-liver aldolase. Biochim. biophys. Acta 85 132, 1964.
15. *DiPietro*, D. L. *Some* aspects of D-fructose metabolism in rat liver. J. biol. Chem. 239 4051. 1964.
16. Drechsler, E. R., Boyer, P. D. & Kowalsky, A. G. The catalytic activity of carboxypeptidase-degraded aldolase. J. biol. Chem. 234 2627. 1959.
17. Farina, F. A., Adelman, R. C., Lo, C. H., Morris, H. P. & Weinhouse, S. Metabolic regulation and enzyme alterations in the Morris hepatoma. Cancer Res. 28 1897. 1968.
18. Fitch, W. M. & Chaikoff, I. L. Extent and *patterns of adaptation of enzyme activities* in livers of normal rats fed diets high in glucose and fructose. J. biol. Chem. 235 554, 1960.
19. Fitch, W. M., Hill, R. & Chaikoff, I. L. Hepatic glycolytic enzyme activities in the alloxan-diabetic rat: response to glucose and fructose feeding. J. biol. Chem. 234 2811. 1959
20. Gracy, R. W., Lacko, A. G., Brox, L. W., Adelman, R. C. & Horecker, B. L. Structural relations in aldolases purified from rat liver and muscl and Novikoff hepatoma. Arch. Biochem. Biophys. 136 480. 1970.
21. Gracy, R. W., Lacko, A. G. & Horecker, B. L. Subunit structure and chemical properties of rabbit liver aldolase. J. biol. Chem. 244 3913, 1969
22. Heinz, F. & Lamprecht, W. Anreicherung und Charakterisierung einer Triosekinase aus Leber. Zur Biochemie des Fructosestoffwechsels. Z. physiol. Chem. 324 88, 1961
23. Heinreich, E., Goldschmidt, S., Lamprecht, W. & Ritzl, F. Z. Der Einfluss von Kohlenhydraten, insbesondre von Fructose auf den Umfang und den zeitlichen Ablauf der Bildung von aktivierter Essigsäure und Brenztraubensäure in der Rattenleber. Z. physiol. Chem. 292 184, 1953
24. Hers, H. G. La fructokinase du foie. Biochim. biophys. Acta 8 416, 1952
25. Hers, H. G. La fructokinase du foie. Role du magnesium et du potassium dans le reaction fructokinasique. Biochim. biophys. Acta 8 424, 1952.
26. Hers, H. G. Le Metabolisme du Fructose, Editions Arscia, Bruxelles, 1957
27. Hers, H. G. & Kusaka, T. Le metabolisme du fructose-1 phosphate dans le foie. Biochim. biophys. Acta 11 427. 1953.
28. Hers, H. G. Triokinase. In: Methods in Enzymology (ed., S. P. Colowick and N. O. Kaplan), V 362, Academic Press, Inc., New York, 1962.
29. Ikehara, Y., Endo, H. & Okada, Y. The identity of the aldolases isolated from rat muscle and primary hepatoma. Arch. Biochem. Biophys. 136 491. 1970.
30. Kaletta-Gmünder, V., Wolf, H. P. & Leuthardt, F. Über Aldolasen. Chromatographische Trennung von 1 Phosphofructaldolase und Diphosphofructaldolase der Leber. Helv. chim. Acta 40 1072, 1957
31. Kirby, S. A., Noda, L. & Lardy, H. A. Adenosinetriphosphate-creatine transphosphorylase. J. biol. Chem. 209 191. 1954.
32. *Kiyasu, J. Y. & Chaikoff, I. L. On the manner* of transport of absorbed fructose. J. biol. Chem. 224 935. 1957
33. Kjerulf-Jensen, A. The phosphate esters formed in the liver tissue of rats and rabbits during assimilation of hexoses and glycerol. Acta physiol. scand. 4 249. 1942.
34. Knox, W. E. & Greengard, O. The regulation of some enzymes of nitrogen metabolism — An introduction to enzyme physiology. In: Advances in Enzyme Regulation (G. Weber, ed.) 3, 247. Pergamon Press, New York, 1965
35. Kowalsky, A. G. & Boyer, P. D. A carboxypeptidase-H_2O^{18} procedure for determination of COOH terminal residues and its application to aldolase. J. biol. Chem. 235 604. 19[illegible]
36. Landau, B. R. & Merlevede, W. Initial reactions in the metabolism of D- and L-glyceraldehyde by rat liver. J. biol. Chem. 238 861. 1963.
37. Lindberg, O. Phosphorylation of glyceraldehyde, glyceric acid and dihydroxyacetone by kidney extracts. Biochim. biophys. Acta 7 349. 1951
38. Mendeloff, A. J. & Weichselbaum, T. E. Role of the human liver in the assimilation of intravenously administered fructose. Metabolism 2 450. 1953.
39. Miller, L. L. Changes in rat liver enzyme activity with acute inanition. Relation of loss of enzyme activity to liver protein loss. J. biol. Chem. 172 113, 1948.

40. Miller M., Drucker W R., Owens, J E Craig, J W & Woodward, H., J Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J clin. Invest. 31 115 1952.

41. Morris, H. P Development, biochemistry and *biology of experimental hepatomas. Advances* in Cancer Research (eds., A. Haddow and S. Weinhouse) 9 227 Academic Press, Inc., New York, 1965.

42. Parks, T E Ben-Gershom, E. & Lardy H. A. Liver fructokinase. J biol. Chem. 227 231 1957

43. Peanasky R. J & Lardy H. A. Bovine liver aldolase. Isolation, crystallization, and some general properties. J biol. Chem. 233 365 1958.

44. Peanasky R. J & Lardy H. A. Bovine liver aldolase. Physical and chemical measurements on the crystalline enzyme J biol. Chem. 233 371, 1958.

45. Pletscher A. Die Fructose. Biologie und Wirkung auf den Alkylalkoholstoffwechsel. Helv med. Acta 20 100, 1953.

46. Reinecke, R. M. The kidney as locus of fructose metabolism. Amer J Physiol. 141 669 1944.

47 Renold, A. E., Hastings, A. B. & Nesbett, F B. Studies on carbohydrate metabolism in rat liver slices. Utilization of fructose and glucose by liver from normal and diabetic animals. J biol. Chem. 209 687 1954.

48. Rutter W J *Aldolase homologues and* metabolic diversification. Abstracts 6th Intl. Cong. Biochem, New York, 1964.

49 Rutter W J Blostein, R. E., Woodfin, B. M. & W ber C. S. Enzyme variants and metabolic diversification. In Advances in Enzyme Regulation (ed G Weber), 1 39 Pergamon Press, New York, 1963.

50. Rutter W J Woodfin, B M. & Blostein, R. Enzymic homology Structural and catalytic differentiation of fructose diphosphate aldolase. Acta chem. scand. 17 S226, 1963.

51. Salomon, L. & Johnson, J E. Transfer of fructose and glucose cross surviving guinea pig intestine. Arch Biochem. Biophys 82 179 1959

52 Sanchez, J J Gonzalez, N S. & Pontis, H. G Fructokinase from rat liver Purification and properties. Biochim. biophys. Acta 227 67 1971

53 Sanchez, J J Gonzalez, N. S. & Pontis, H. G. Fructokinase from rat liver The role of K^+ on the enzyme activity Biochim. biophys. Acta 227 79 1971

54. Schapira, F L activite fructose-1-phosphoaldolasique des tissus de mammiferes. Etude des activities aldolasiques du foie. Bull. Soc. Chim. biol. 43 1367 1961.

55 Schapira, F Dreyfus, J -C. & Schapira, G Anomaly of aldolase in primary liver cancer Nature 200 995, 1963.

56. Sharma, C., Manjeshwar R. & Weinhouse, S. Effects of diet and insulin on glucose-ATP phosphotransferases of rat liver J biol. Chem. 238 3840 1963.

57 Sharma, R. M., Sharma, C., Donnelly A. J Morris, H. P & Weinhouse, S. Glucose-ATP phosphotransferases during hepatocarcinogenesis. *Cancer Res. 25 193, 1965.*

58. Shatton, J B., Donnelly A. J & Weinhouse, S. Glucokinase activity and glycogen levels during hepatocarcinogenesis by azo dyes. Cancer Res. 22 1372, 1962.

59. Slein, M. W Some studies of fructose metabolism. In Phosphorus Metabolism (eds., W D McElroy and B. Glass) 1 98, John Hopkins Press, Baltimore, 1951

60 Slein, M. W Cori, G T & Cori, C. F A comparative study of hexokinase from yeast and animal tissues. J biol. Chem. 186 763, 1950.

61 Sols, A. & Crane, R. K. S bstrate specificity of brain hexokinase. J biol. Chem. 210 581, 1954.

62. Spiro, R. G & Hastings, A. B. Studies on carbohydrate metabolism in rat liver slices. XI. Effect of prolonged insulin administration to the alloxan-diabetic animal. J biol. Chem. 230 751 1958.

63 Spolter P D Adelman, R. C. & Weinhouse, S. Distinctive properties of native and carboxypeptidase-treated aldolases of rabbit muscl and liver J biol. Chem. 240 1327 1965

64. Sugimura, T Matsushima, T Kawachi, T Hirata, Y & Kawabe, S.. Biological and biochemical evaluation of malignancy in experimental hepatomas. Gann, Monograph 1 143, 1966.

65. Sweeney M. J Ashmore, J Morris, H. P & Weber G.. Comparative biochemistry of hepatomas. IV Isotope studies of glucose and fructose metabolism in liver tumors of different growth rates. Cancer Res. 23 995, 1963.

66. Tanko, B. & Robison, R. The hydrolysis of hexosediphosphoric ester by bone phosphatase. The isolation of pure fructose-1-phosphate. Biochem. J 29 961, 1935.

67 Vestling, C. S., Mylrole, A. K., Irish, V & Grant, N H. Rat liver fructokinase. J biol. Chem. 185 789 1950.

68. Walker D. G The postnatal development of hepatic fructokinase. Biochem. J 87 576, 1963.

69 Weber G Behavior of liver enzymes in hepatocarcinogenesis. In Advances in Cancer Research (eds., A. Haddow and S. Weinhouse), 6 403, Academic Press, Inc. New York, 1961.

70. Weber G The molecular correlation concept Studies on the metabolic pattern of hepatomas. Gann, Monograph 1 151, 1966.

71. W ber G. & Ashmore, J Absent fructose-1,6-diphosphatase activity in hepatomas. Exp. Cell Res. 14 226, 1958.

72. Weber G & Cantero, A. Glucose-6-phosphatase activity in normal, precancerous, and neoplastic tissues. Cancer Res. 18 105, 1955

73. Weber G & Morris, H. P Comparative biochemistry of hepatomas. III Carbohydrate enzymes in liver tumors of different growth rates. Cancer Res. 23 987 1963.

74. Weichselbaum, T E., Margraf, H. W & Elman, R. Metabolism of intravenously infused fructose in man. Metabolism 2 434, 1953

75. Weinhouse, S. Glycolysis, respiration and enzyme deletions in slow growing hepatic tumors. Gann, Monograph 1 99 1966.

76. Wyshak, G H. & Chaikoff, I. L. Metabolic defects in the liver of fasted rats as shown by utilization of ^{14}C labeled glucose and fructose. J biol. Chem. 200 851 1953.

Acta med. scand. Suppl. 542, pp. 57—64

ASPECTS OF FRUCTOSE METABOLISM IN NORMAL MAN

J Bergström, P Fürst, F Gallyas, E. Hultman, L. Hᴣon Nilsson, A. E. Roch Norlund & E. Vinnars

From the Renal Clinic the Clinical Central Laboratory and the Department f Anesthesiology St. Eriks Sjukhus, Stockholm, Sweden

Abstract. In normal subjects infusion of fructose (1 g/kg/hr) for 4 hrs resulted in an increase in the glycogen content of the *m. quadriceps femoris* of 3.3 g/kg wet muscl (muscle samples obtained by needle biopsy). This was equal to the amount f glycogen formed after glucose infusion f the same magnitude. In muscl depleted of glycogen by exercise, infusion of glucose resulted in twice as much glycogen formed, as did fructose infusion. Formation f liver glycogen was much higher after fructose than after glucose infusion (liver samples obtained by Menghini biopsy) Studies by hepatic vein catheterization indicated that glucose formation by the liver was insufficient to account for the synthesis of muscl glycogen, which presumably occurred directly from fructose taken up by the muscle.

The occurrence of high lactic acid concentra tions in blood resulting from infusion of fructose could be partly abolished by simultaneous infusion of amino acids, thus lessening the risk of lactic acidosis.

In 1874, Külz (16) first reported that diabetic patients were able to use fructose administered orally better than other sugars. Since this time many studies have been performed with the purpose of establishing how fructose is utilized and metabolized in man. This is of particular interest as fructose is widely used for parenteral nutrition with carbohy drate.

For this purpose fructose is stated to have several advantages over glucose (26). Fructose is more rapidly utilized than glucose resulting in a lower total blood sugar con centration, when equivalent amounts of ei ther are given (4, 20 29) It has been reported that postoperative and uremic pa tients with glucose intolerance can utilize fructose in a normal manner (10 17) Since fructose is not dependent upon insulin for intracellular penetration, it has been consid ered to be of value in cases of diabetic acidosis and/or after total pancreatectomy (9 21). Finally fructose is stated to have a less irritat ing effect upon the peripheral veins than does glucose, which implies that solutions for intravenous infusion can be given in much higher concentrations (26).

It has been known for almost two decades that fructose administered intravenously is rapidly taken up by the liver and partly metabolized to lactic acid which is subsequently released into the general circulation

(20). The clinical implication of this was pointed out by Bergström et al., in 1967 (5) who demonstrated that this formed lactic acid could give rise to pronounced metabolic acidosis which could be potentially dangerous in patients with a disturbed acid-base balance. This was confirmed by Andersson et al (2) who reported that fatal acidosis could ensue following massive intravenous administration of fructose to children with metabolic acidosis.

In addition to accumulation of lactate, a significant elevation in the plasma levels and the urinary excretion of uric acid was demonstrated in man following rapid administration of fructose (25) This was ascribed to rapid depletion of ATP in the liver resulting from phosphorylation of fructose (19) to fructose-1 P (6 32).

Oral feeding of fructose or sucrose has been reported to produce hyperlipemia in man, which has been implicated in the etiology of occlusive arteriosclerotic disease (e.g 23 33). The fructose portion of sucrose appears to be responsible for the hyperlipemic effect.

Our group at S:t Erik s Hospital has studied the formation and breakdown of glycogen in human muscle tissue (quadriceps femoris) with the needle biopsy technique (3 14) More recently similar studies of liver glycogen in normal man have been performed. The Menghini technique for liver biopsy was used. We have also applied the technique of liver vein catheterization with measurement of splanchnic blood flow with BSP or cardiogreen, and determination of metabolites in arterial and hepatic venous blood.

In the following are briefly reported some results in connection with fructose administration. Some recent data on the modification of lactate formation by simultaneous intravenous administration of amino acids will also be presented.

Formation of muscle and liver glycogen after glucose and fructose infusions was studied in normal healthy volunteers. Glucose or fructose were administered intravenously as a constant infusion (20 % solution) 1 g/kg body weight per hour during approximately 4 hours. In the first experiments only muscle biopsies were obtained before and after the infusion (4). In later studies (24) liver biopsies were also obtained before and after administration of fructose (3 subjects) or glucose (7 subjects). These results are summarized in Fig 1 From these it can be seen that in resting human subjects following an overnight fast, there is a decrease in liver glycogen content during a further 4 hours' starvation. If an infusion

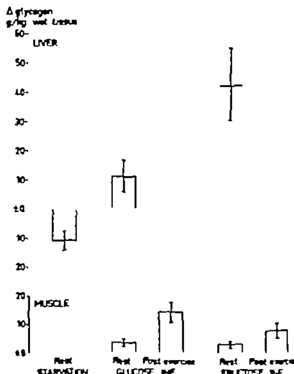


Fig 1 Changes in liver and muscle glycogen during fructose or glucose infusion (1 g/kg b.w./4 hrs). For liver glycogen the columns denote changes from values obtained after an overnight fast. A decrease in liver glycogen content was observed during further 4 hours' starvation. Increases in liver glycogen were found when fructose or glucose (1 g/kg b.w./hour) were given during 4 hours. Muscle glycogen was determined before and after an infusion of fructose or glucose at rest or following heavy exercise with one leg.

of fructose or glucose is given over a corresponding period of time, there is a considerable increase in the liver glycogen content. The increase was found to be much higher with fructose than with glucose, indicating that the former is more efficiently utilized for glycogen formation in the liver than is the latter. This is in agreement with earlier studies made by Cori (7) who demonstrated that fructose was more rapidly metabolized in the rat liver than was glucose.

In muscle, on the other hand, the rate at which glycogen formation occurs following administration of either fructose or glucose, seems to be primarily dependent upon whether the muscle glycogen level is normal or has been lowered by exercise. In resting subjects with a normal muscle glycogen content formation of glycogen, following either fructose or glucose infusion, was on average the same. However in muscle previously depleted of glycogen by heavy exercise (in all these experiments with only one leg) glycogen resynthesis after glucose infusion was about twice as fast as after fructose infusion. This difference was highly significant. The higher rate of glycogen formation following glycogen depletion by heavy exercise may at least be partly explained by the transformation of glycogen synthetase from the D to the I form (the I form being considered to be the active form *in vivo*) (8). The discrepancy between the results obtained with fructose and glucose may be due to a) the higher stimulation of insulin release during the glucose infusion, and to b) the lower total supply of carbohydrate to the muscles by the blood stream during the fructose infusion.

A very interesting problem is whether fructose itself is taken up by the muscle tissue and is used in the synthesis of glycogen or whether it must first be metabolized in the liver or in some other tissue, to glucose or other substrate. Earlier studies in man and experimental animals have yielded conflicting results.

From experiments performed with isolated rat diaphragm it has been shown that fructose is taken up from the medium and assimilated to glycogen. Glycogen formation from fructose, however is much less than from an equivalent amount of glucose. The presence of glucose in the medium decreased the uptake of fructose, whereas an excess of fructose, on the other hand, did not interfere with glucose uptake to any extent (18 22). As glucose is always present in the intact organism it has, thus, been assumed that under physiological conditions fructose is not phosphorylated (13). Contrary to this, studies of peripheral arteriovenous difference of fructose, during infusion of fructose, have indicated that in both man and dog there is an actual uptake of fructose presumably by the skeletal muscle tissue (15, 30 31).

We approached this problem indirectly by measuring the output of glucose from the splanchnic area during a fructose infusion. One of these experiments is illustrated in Fig 2. In this it can be seen that a fructose infusion caused a decrease in the production of glucose from the splanchnic area, a negative v-a difference being recorded during part of the infusion. As this occurred in spite of a moderate increase in blood glucose concentration, it indicates that peripheral glucose consumption diminished more rapidly than glucose production from the liver. Similar results have been obtained in other experiments (4), *cf* also Fig 4.

In the experiment illustrated in Fig 2 it can also clearly be seen that the main source of lactate during fructose infusion is the liver which rapidly removes fructose from the arterial blood.

One explanation of these results may be that lactate formed in the liver is taken up by the muscle and metabolized to glycogen by gluconeogenesis. This, however is unlikely since it is known that the gluconeogenetic pathway is absent or of low capacity in skeletal muscle. Furthermore the amount of lactate formed during the infusion period

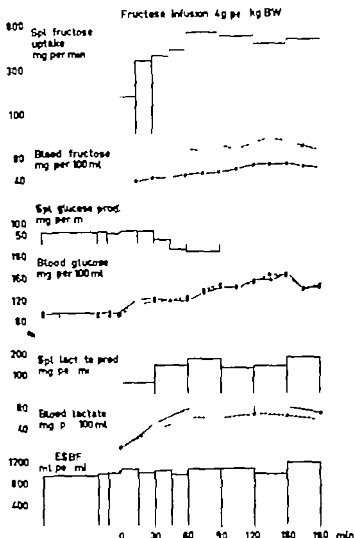


Fig Estimated splanchnic blood flow (ESBF) and splanchnic metabolism before and during infusion f 20 fructose to a normal subject.
rterial blood
——— liver vein blood

(25 g) was insufficient to account for the total increase in muscle glycogen (60 g assuming a muscle mass of 20 kg) especially when considering that about 20 g were present in the body fluids (assuming an even distribution in a total body water volume of 35 kg).

In the experiment illustrated in Fig 2 a catheter was also placed in the renal vein and the renal glucose uptake/production was calculated from the renal blood flow (determined by the PAH-method) and the v-a difference for glucose. During the infusion period fructose was taken up by the kidney (and excreted in the urine) During the later part of infusion the renal v a difference for glucose was positive, indicating that the kidney was producing glucose. The total production of glucose during the fructose infusion, however did not amount to more than 6 g a very small amount compared to the total amount of muscle glycogen formed.

LACTATE CONC mmol/L
10
8
FRUCTOSE FRUCTOSE AMINO ACIDS
6
4
2
0

Fig 3 Blood lactate concentration after infusion of fructose (1 g/kg b.w) or fructose + amino acids (1 g/kg b.w + 50 mg N/kg b.w) during 60 min.

From this and other similar experiments it was concluded that skeletal muscle of man can utilize fructose directly from the blood stream and assimilate it to glycogen. The way in which fructose is metabolized in human skeletal muscle remains obscure. In preliminary experiments we have measured the levels of the hexose phosphates (G-6-P G-1 P F-6-P FDP) and triose phosphates (dihydroxyacetone-P glyceraldehyde-3-P) in skeletal muscle and found only slight increases or no increases in concentration during a fructose infusion. Similar results were obtained when glucose was infused which resulted in pronounced glycogen formation (unpublished observations) Fructose-1 P which is known to increase to very high levels in liver due to phosphorylation of fructose by ketohexokinase was not measured in the muscle samples. However ketohexokinase has not been found to be present in rat skeletal muscle (1).

It has been shown that amino acid solutions given intravenously are better utilized if a carbohydrate is also given simultaneously and that fructose appears superior to glucose in favouring amino acid utilization 11 12).

In recent experiments we have studied the effect of amino acid administration on fructose metabolism. The solution used consisted of the eight essential amino acids in the mutual proportions recommended by Rose + the non-essential amino acids alanine arginine, glycine serine proline, histidine and aspartic acid, cf Vinnars (27) Table II, group 2 We found that by combining the fructose infusion (1 g/kg/hour) with an infusion of this amino acid solution (0.05 g N/kg/hour) the level of lactate in the blood was much lower than when only fructose was given (Fig 3)

Proceeding from this observation we recently made a few studies with liver vein catheterization during fructose infusion together with amino acids. A typical study performed in a normal man is illustrated in Fig 4 Uptake of fructose over the splanchnic area was high throughout the entire infusion period and did not change appreciably when the amino acids were given simultaneously during the last hour Splanchnic glucose production fell during fructose infusion as observed in earlier experiments, but increased again when amino acids were administered. The most striking changes were observed in lactic acid production. This, as seen earlier was high during the fructose infusion period, but decreased considerably on addition of the amino acids resulting in a rapid decrease in the blood lactate concentration. This pronounced effect of the amino acids on the production of lactate from fructose by the liver is of considerable interest, since it indicates that fructose can be given with less danger of acidosis from excessive lactate formation, if an excess of amino acids is included in the infusate.

The mechanism(s) through which the above phenomena are brought about remain obscure. One possibility is inhibition of lactate formation through blocking of some

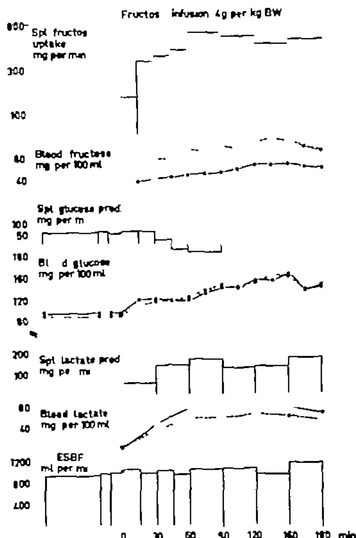


Fig Estimated splanchnic blood flow (ESBF) and splanchnic metabolism before and during infusion f 20 fructose to a normal subject.
rterial blood
——— liver vein blood

(25 g) was insufficient to account for the total increase in muscle glycogen (60 g, assuming a muscle mass of 20 kg) especially when considering that about 20 g were present in the body fluids (assuming an even distribution in a total body water volume of 35 kg).

In the experiment illustrated in Fig 2 a catheter was also placed in the renal vein and the renal glucose uptake/production was calculated from the renal blood flow (determined by the PAH method) and the v-a difference for glucose. During the infusion period fructose was taken up by the kidney (and excreted in the urine) During the later part of infusion the renal v a difference for glucose was positive indicating that the kidney was producing glucose. The total production of glucose during the fructose infusion, however did not amount to more than 6 g a very small amount compared to the total amount of muscle glycogen formed.

REFERENCES

1. Adelman, R. C., Ballard, F J & Weinhouse, S. Purification and properties f rat li er fructokinase. J biol. Chem. 4. 3350 196
2. Andersson, G Brohult, J & Sterner G Increasing metabolic acidosis follow ng fru tose infusion in two children. Acta paedi t scand. 58 301 1968.
3. Bergstrom, J Muscle electrolytes in m. Determined by neutron activation an u ns n needle biopsy specimens. A study normal subjects, kidney patients, nd pati t with chronic diarrhoea. Scand. J clin Lab In est 14, Suppl. 68, 110 pp, 1962
4. Bergström, J & Hultman, E Syr cns f muscle glycogen in man after gl cose and fructose infusion. Acta med sc d 18 93, 1967
5. Bergström, J Hultman, E. & Roch Norlund, A. E. Lactic acid accumulation in co ne t on with fructose infusion. Acta med sc d 184 359 1968.
6. Burch, H. B Max, P J Chyu K & Lowry O H. Metabolic intermediates li f rats given large amounts f fructose d hydroxyacetone. Biochem biophys R Com mun. 34 619, 1969
7. Cori, C. F The fate of sugar in th animal body III. The rate of glycogen formation in the liver of normal and insulinized rats during th absorption of glucose fructose and galactose. J biol. Chem. 70 577 1926
8. Danforth, W H. Glycogen synth tase ct ity in skeletal muscle J biol Chem 240 588, 1965.
9. Daughaday W H. & Weichselbaum, T E Utilization of i.v fructose in diabetic acidosis and in a pancreatectomized human Metabolism 2 459 1953.
10. Drucker W R., Miller M Craig, J Jeffries, W M., Levey S. & Abbott, W E A comparison of the effect f operation on glucose and fructose metabolism. Surg. Forum 3 548, 1953.
11. Elman, R. Time factors in the utilization of mixture of amino acids (protein hydrolysate) and dextrose given intravenously J clin. Nutr 1 287 1952—53
12. Elman, R., Pareira, M D., Conrad, E J Weichselbaum, T E., Moncrief, J A & Wren, C. The metabolism of fructose as related to the utilization of amino acids when both are given by intravenous infusion. Ann. Surg. 136 635, 1952.
13. Hers, H. G The conversion of fructose-1-C14 and sorbitol 1-C14 to liver and muscle glycogen in the rat. J biol. Chem. 214 373, 1955.
14. Hultman, E. Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. Scand. J clin. Lab Invest. 19, Suppl. 94 63 pp, 1967
15. van Itallie, B., Morgan, M. C., Cathcart, R. T Leduc, G G & Dotti, L. B. Peripheral assimilation of fructose in man. Proc. Soc. exp. Biol. (N Y.) 84 713, 1953.
16. Kulz, E. Beitrage zur Pathologie und Therapie des Diabetes Mellitus. Marburg, 1874 p. 1 0
17. Luke, R. G Dinwoodie, A. J Linton, A. L. & Kennedy A. C. Fructose and glucose tolerance in uremia. J Lab. clin. Med. 64 731 1964.
18. Mackler B. & Guest, G M. Effects of insulin and gl cose on utilization f fructose by isol ted rat diaphragm. Proc. Soc. exp. Biol. (N Y) 83 327 1953.
19. Maenpää, P H., Raivio, K. O & Kekomäki, M. P Liver adenine nucleotides fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.
20. Mendeloff, A. I. & Weichselbaum, T E. Role f th human liver in the assimilation of intravenously administered fructose. Metabolism 2 450, 1953.
21. Miller M Drucker W R., Owen, J E., Craig, J W & Woodward, H. Metabolism of intravenous fructose and glucose in normal nd diabeti subjects. J clin. Invest. 31 115, 1952.
22. Moriwaki, T & Landau, B. R. Fructose and glucose metabolism in the normal and diabetic rat diaphragm in vitro. Arch. Biochem. 98 544, 1962.
23. Nikkilä, E. A. & Pelkonen, R. E... of alimentary hyperglycemia by ... and glycerol in man. Proc. Soc. exp. Biol. (N Y.) 123 91 1966.
24. Nilsson, L. H..son & Hultman, E. Liver glycogen in man the effect of total ... different diets and of glucose and ... infusions. Paper presented at ... Congr Gastroenterology Copenhagen, ... July 1970.
25. Perheentupa, J & Raivio, K. ...-induced hyperuricaemia. Lancet ...
26. Thorén, L. Parenteral ... hydrate and alcohol. Acta ... 325 75, 1964.
27. Vinnars, E. Effect of intravenous ...

administration on nitrogen retention. Scand. J clin. Lab. Invest. Suppl. 117 Vol. 27 1971
28. Weber G Regulation of pyruvate kinase. Adv Enzyme Regul. 7 15 1969
29. Weichselbaum, T E., Elman, R. & Lund, R. H. Comparative utilization f fructose and glucose given intravenously Proc. Soc. exp. Biol. (N Y) 75 816, 1950.
30. Weichselbaum, T E., Margraf, H. W & Elman, R. Metabolism of intravenously infused fructose in man. Metabolism 2 434, 1953.
31. Weinstein, J J & Roe, J H. The utilization of fructose by human subjects and animals. J Lab. clin. Med. 40 39 1952.
32. Woods, H. F Eggleston, L. V & Krebs, H. A. The cause of hepatic accumulation of fructose 1-phosphate on fructose loading. Biochem. J. 119 501 1970.
33. Yudkin, J & Roddy J Levels of sucrose in patients with occlusive artheriosclerotic disease. Lancet ii 6, 1964.

Acta med. scand. Suppl. 542, pp. 65—75

HEREDITARY FRUCTOSE INTOLERA[illegible]

J. Perheentupa, K. O. Raivio and E. A. [illegible]kila

From Children's Hospital, and Third Department [illegible] Medic[illegible]
University of Helsinki, 00290 Helsinki 29, F[illegible]d

Abstract. The clinical and metabolic aspects of hereditary fructose intolerance (HFI) are reviewed and some new observations on children with HFI are reported. The acute rise in plasma FFA level which was earlier shown to follow an acute exposure to fructose in these patients, was found to be associated with an increase in [illegible] excretion and a decrease in plasma TG level. Abolition of the fructose induced hypoglycemia by an infusion of glucose after injection of fructose prevented the rise in plasma FFA, but did not modify the increase in plasma lactate or urate concentration. Maintaining normal or high plasma inorganic phosphate levels after fructose injection by infusion of phosphate buffer did not alter the fructose-induced changes in blood glucose or lactate, or in plasma FFA or urate concentration. The hyperuricemia and hyperuricosuria after fructose were larger in HFI patients than in normal children. Administration of fructose in the small doses of 0.33—1.3 g/kg daily for 3—5 days brought about [illegible] gradual fall in plasma cholesterol level, and a rise in the excretion of urate and 11-ketogenic steroids, but no increase in [illegible].

Abbreviations: ATP, adenosine triphosphate; F 1-P, fructose-1-phosphate; FFA, unesterified fatty acids; HFI, hereditary fructose intolerance; 11 KGS, 11-ketogenic steroids; P, inorganic phosphate; TG, triglyceride.

5

Several common dietary [illegible] actually poisonous to individuals [illegible] afflicted by a genetic defect [illegible] pairment in the metabolism [illegible] pound. Hereditary fructose [illegible] 17) is an example of th[illegible] but unlike many condition[illegible] sion of the harmful subst[illegible] acid very difficult (e.g. [illegible] metabolism), HFI [illegible] and a fact, aversion to [illegible] The develops in the [illegible] fructose-free diet [illegible] healthy life person may lead an [illegible] and be totally unaware of the [illegible] practice of [illegible] of his disease. The [illegible] nutrition may using fructose for [illegible] individual [illegible] the risk the life of such an individual [illegible] On the other course of some incidental illness [illegible] er hand many infants with HFI die [illegible] disease as they are unable to [illegible] feeding and as the [illegible] causing the [illegible] ly recognized by a [illegible] specifically altered

HFI is apparently not very rare. About one hundred cases have been reported in the literature, many more have been diagnosed in several countries, and most of the patients probably still remain unrecognized. In Finland, we know of 18 patients belonging to 14 families.

DIFFERENT CLINICAL MANIFESTATIONS

The severity of the clinical presentation of HFI depends on the intake of fructose: no symptoms are associated with the metabolic anomaly in the absence of dietary fructose. Young infants tend to have a chronic serious disease, usually dominated by signs of liver damage. After the first year of life, periodic vomiting may occur which will probably be labelled as acetonemic (7). Accidental deviations from the self imposed dietary restrictions may cause attacks of abdominal pain, nausea, vomiting and hypoglycemic symptoms. In adults renal calculi (22), polyuria and periodic or progressive weakness or paralysis (31) have been reported as results of chronic renal tubular dysfunction and hypokalemia. An enlarged liver is common to all forms of presentation in infancy and childhood, but may not be notable in adults (18).

The infantile symptomatology deserves some elaboration. Poor appetite and vomiting with resulting slow gain or even loss of weight and hypochromic anemia are the most regular features. The vomiting varies from projectile to spitting. Thus, the clinical picture varies from one of failure to thrive to one of pyloric stenosis. Periodic symptoms of hypoglycemia may add to the picture frequently as apneic spells. Icterus is common, especially prolongation of neonatal jaundice, and more serious signs of liver damage may develop: gross hepatomegaly, hypoalbuminemia and abdominal distension with ascites. The spleen is frequently enlarged. Deficiency of hepatic coagulation factors is also common, and hemorrhage tendency may dominate the picture with large gastrointestinal or cerebral bleeding (49). The urine usually contains protein and increased amounts of amino acids. Metabolic acidosis may be severe: it is partly of renal tubular origin but hyperlactatemia (9, 44) is a contributing factor. If plasma amino acids are analyzed, large elevations in the concentrations of tyrosine and methionine may be found and the condition may be called acute tyrosinosis (20, 28, 54). Infantile HFI may thus appear under a variety of diagnostic labels, partly depending upon the availability of laboratory facilities.

Some infants may be able at the age of a few months to convey their distaste to sweet tasting foods to an intelligent mother and remain healthy even after weaning from breast. Any infant with an enlarged liver and protein- or aminoaciduria is so suspect of having HFI that fructose should be excluded from the diet, until the diagnosis has been ruled out. *Similarly parenteral fructose* ought not be given to any patient not positively known to tolerate sugar.

ENZYME DEFECT AND SECONDARY METABOLIC DISTURBANCES

The basic defect in HFI is a gross deficiency in the activity of aldolase B, also called liver aldolase (Fig. 1) (21, 32, 35, 38, 39, 52) which is normally present in the liver, renal cortex and intestinal mucosa. This enzyme provides practically all of the fructose-1-phosphate splitting activity and also most of the fructose-1, 6-diphosphate splitting activity of these organs. The rest of the latter activity is due to a different molecule, aldolase A or muscle aldolase (42). The mutation probably involves the structural gene for the B aldolase. This is suggested by the demonstration in some patients of material that crossreacts with antibody to normal B aldolase but has little measurable enzyme activity (40).

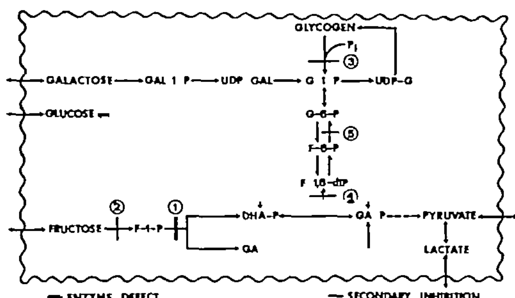
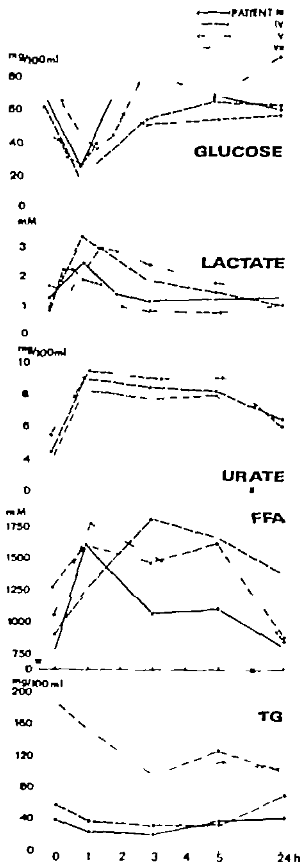


Fig. 1 Metabolism of fructose in the liver cell. The enzyme deficient in hereditary fructose intolerance is indicated by 1. Secondary inhibition of metabolic steps by accumulating F 1 P is indicated by 2 (fruct kinase), 3 (phosphorolysis of glycogen) 4 (synthesis of F 1, 6-diP by aldolase), and 5 (conversion of F-6-P to G-6-P by phosphohexose isomerase).
Abbreviations F fructose G glucose Gal, galactose P phosphate P_i inorganic P UDP uridine diphosphate DHA, dihydroxyacetone GA, glyceraldehyde.

The immediate metabolic disturbance caused by fructose in absence of adequate F 1 P aldolase activity is accumulation of the substrate F 1 P in the three organs, as they also possess fructokinase and are thus able to phosphorylate fructose rapidly Interestingly F 1 P probably accumulates briefly even in the normal organism after a fructose load (4, 56), and many of the metabolic consequences of fructose in HFI can be regarded as marked exaggerations of events taking place in normal individuals. Some of these metabolic consequences are effects of the accumulating F 1 P some are due to sequestration of inorganic phosphate as F 1 P and resulting intracellular deficiency of P_i. Temporary depletion of ATP is probably a further factor The acute functional derangements in organs other than the primary three are probably mediated by hypoglycemia (Fig 2) or acidosis.

Increased utilization of glucose due to increased insulin activity is not responsible for the fructose-induced hypoglycemia in HFI. This is shown by the decrease of plasma insulin levels (9 18) and by the increase of plasma FFA concentration (9 37) after fructose administration. The mechanism of the marked hypoglycemic response, which after large doses may result in zero blood glucose level, is inhibition of hepatic output of glucose. This has been shown by injecting a tracer dose of radioactive glucose and demonstrating a decrease in the rate of fall of blood glucose specific radioactivity when fructose is injected (14) This indicates that the production of glucose is inhibited both from liver glycogen (as shown also by the lack of glucagon response during the fructose effect (9 18 44)) and from gluconeogenic sources. An inhibition of glucose-6-phosphatase would explain both, but such a block obviously is not



present since galactose is effectively converted to glucose and is able to relieve fructose-induced hypoglycemia (9 19 50) In fact, this finding indicates that the inhibition of glycogen breakdown must take place at or above the phosphorylase reaction. Depletion of the liver cell of P_i, which is needed in the phosphorolysis, is one possible mechanism, particularly as the sequestration of phosphate is manifested as a fall in plasma phosphate concentration even before the hypoglycemic response sets in. However the fructose-induced hypoglycemia is not influenced by phosphate infusion which prevents the depletion of P_i (Fig. 4 ref 11 12)

Two factors are known which probably contribute to the inhibition of hepatic gluconeogenesis. Aldolase A, which provides practically all the fructose-1 6-diphosphate splitting and building activity in the absence of active aldolase B in HFI is inhibited by the accumulating F 1 P (16) (Fig 1) The conversion of fructose-6-P to glucose-6-P by phosphohexose isomerase has also been reported to be inhibited by F 1 P (57) Depletion of ATP may also be involved

Blood lactate concentration rises after fructose load in HFI patients as sharply as in normal subjects (Fig. 2, Table 1) In normal subject lactate is thought to be derived directly from fructose, which enters the glycolytic pathway more rapidly and to a greater extent than glucose. The immediate source of the excess lactate and the mechanism of its generation in HFI are unknown, but it seems unlikely that the metabolic path-

Fig 2 Blood glucose and lactate, plasma urate, unesterified fatty acid (FFA) and triglyceride (TG) concentrations in 4 children with hereditary fructose intolerance following an oral fructose load. After an overnight fast, 0 4 g/kg (0.7 g/kg in case III) of fructose was ingested and food was withheld for the next 5 hours. The last blood sample was drawn after a overnight fast. Glucose, lactate and urate were determined with specific enzymatic methods FFA and TG according to ref. 83 and 6.

way from fructose to lactate is similar in normal and HFI subjects. Neither is the lactate production dependent on the hypoglycemia, since fructose injection is followed by hyperlactatemia also if normoglycemia is maintained by glucose infusion (Fig 4).

The plasma FFA concentration rises sharply in patients with HFI after the administration of fructose (Fig 2, ref. 37). This rise seems to be dependent on the hypoglycemia as it can be inhibited by glucose infusion (Fig 4). It is probably evoked by increased lipolysis of adipose tissue triglycerides resulting from hypoglycemia induced release of epinephrine (Fig 3), corticotropin (as reflected by an increase in the excretion of 17 KGS, Fig 3) and growth hormone (9 23). Another factor which might be thought to contribute to the rise of plasma FFA is decreased re-esterification in adipose tissue caused by hypoglycemia and concomitant decrease of α-glycerophosphate (37). This mechanism is less plausible, however since fructose is readily metabolized in adipose tissue (15) also in HFI and provides α-glycerophosphate. The production of ketone bodies is enhanced by administration of fructose in HFI (7 44) in analogy with other situations associated with increased mobilization of FFA.

Normally the liver extracts FFA in pro-

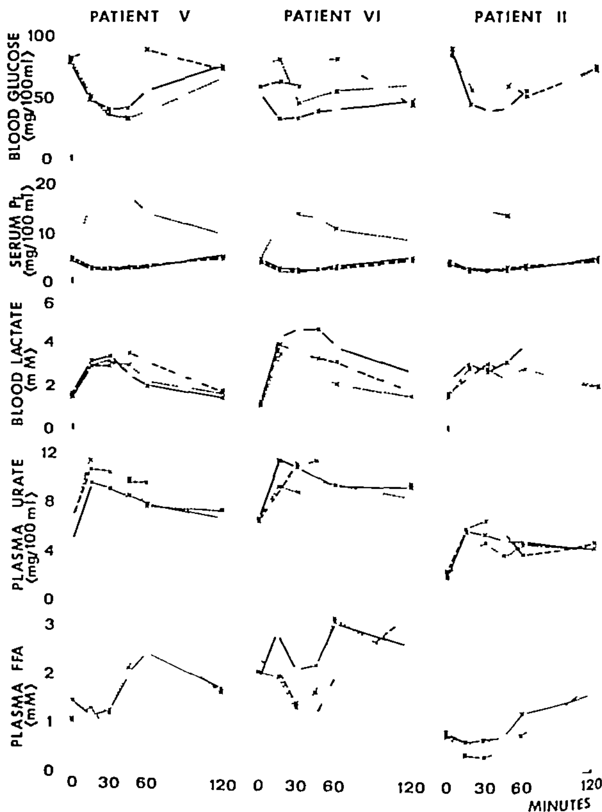


Fig 3. Urinary excretion of urate, epinephrine (10) and 17 ketogenic steroids (17 KGS) (3) in 4 children with hereditary fructose intolerance during control periods and after fructose administration as described in Fig. 2.

Table I. *Maximal changes measured in blood glucose and lactate, and in plasma urate concentration after fructose in 5 normal children and in 5 patients with hereditary fructose intolerance*

	Normal children		HFI patients	
	Mean	Range	Mean	Range
Urate mg/100 ml	+ 2.8	1.9— 4.5	+ 4.5	3.8— 5.0
per cent	+ 82	65— 168	+ 115	77— 181
Lactate mg/100 ml	+22.6	21.3—24.5	+21.5	10.9—30.4
per cent	+ 149	112— 175	+ 188	74— 287
Glucose mg/100 ml	+ 25	13— 32	— 39	—20— —49
per cent	+ 39	20— 96	— 55	—38— —72

The dose wa 0.5 g/kg in the normal children and 0.4 g/kg in the HFI patients, given as a 2 min intravenous injection. All measurements were done with specific enzymatic methods.


PATIENT V
PATIENT VI
PATIENT II
BLOOD GLUCOSE (mg/100ml)
100
50
0
SERUM Pi (mg/100 ml)
20
10
0
BLOOD LACTATE (m M)
6
4
2
0
PLASMA URATE (mg/100 ml)
12
8
4
0
PLASMA FFA (mM)
3
2
1
0
0 30 60 120
0 30 60 120
0 30 60 120
MINUTES

portion to their concentration in plasma and rapidly esterifies them to triglycerides and phospholipids, which are released into the circulation in the form of lipoproteins. Increased plasma FFA concentration induced by epinephrine injection is associated with a rise in plasma TG concentration in normal subjects (for a review see 36) In contrast, the rise of plasma FFA after fructose in HFI is associated with a fall in plasma TG concentration (Fig 2) This may be due to impairment of the hepatic uptake or esterification of FFA, or due to an inhibition of release of TG or a combination of these factors. Since both glucose and insulin are known to stimulate plasma TG production it is possible that hypoglycemia and low plasma insulin level are responsible for the acute decrease of plasma TG after fructose. The fact that heavy accumulation of fat in the liver is a very characteristic histologic finding in HFI patients exposed to fructose (24, 41 44) suggests that the release of TG from the liver is inhibited. In contrast to TG which in the fasting state originate solely from the liver the plasma cholesterol concentration showed no consistent alteration during the acute exposures to fructose. During prolonged exposures to small amounts of fructose, the plasma cholesterol level fell gradually in all patients studied (Table 2), whereas fasting plasma TG and FFA showed no change. The lowering of the cholesterol concentration could be due to a direct effect of fructose on the liver and/or intestinal mucosa.

Fig 4. Blood glucose and lactate, plasma inorganic phosphate (P), urate and unesterified fatty acid (FFA) concentrations in 3 patients with hereditary fructose intolerance following an i.v load of fructose alone (———) fructose with glucose (x x) and fructose with phosphate (x x) 0 4 g/kg of fructose was injected in 2 min as 20 / solution after an overnight fast. On another day the injection was followed by 60 min infusion of 10 / glucose, 0.14 g per kg and h. On yet another day the fructose injection was followed by a 45 min infusion f 0.15 M Na-phosphate buffer corresponding to 1 5 mmoles of P per kg and 45 min.

The observation of a heavy urate sediment in the urine of HFI patients during and after exposure to fructose led us to the discovery that urate production is increased by fructose both in HFI patients and in normal subjects (45) The mechanism behind this effect was subsequently shown to be an increased breakdown of liver adenine nucleotides (30 47), apparently induced by the depletion of P in the liver cell. As the accumulation of F 1 P and hence the sequestration of P are thought to be more marked and more prolonged in subjects with HFI than in normal individuals, one would except the hyperuricemic effect of fructose to be more marked in HFI, too. Our data (Table I) and those of others (34) indicate that this difference is present even though it is not large. This is further supported by the observation that a prolonged exposure to fructose causes a significant increase in urinary urate excretion in HFI patients (Table II), but not in normal subjects. The fructose-induced hyperuricemia in HFI patients is not prevented by phosphate or glucose infusions (Fig 4)

Vomiting and nausea are characteristically experienced by HFI patients after oral intake of fructose. It is likely that these symptoms are associated with the enzyme defect and F 1 P accumulation in the intestinal mucosa. Fructose seems to impair the absorption of glucose in affected subjects (19 51). There are also some observations suggesting that diarrhea is a component of the HFI syndrome (5 18 25), though inconstant.

Morris (33 34) has shown that acute loads of fructose impair the reabsorption of bicarbonate, phosphate, amino acids and urate in the proximal tubule. The condition is similar to the tubular disturbance known as the Fanconi syndrome, associated e.g with cys-

Table II. *Effect of prolonged exposure to fructose on the plasma cholesterol concentration and the urinary excretion of urate and 17 ketogenic steroids in three children with hereditary fructose intolerance*

	Case III	Case IV	Case VII
Daily dose of fructose (g/kg)	0 65—1.30	0 43—0 60	0.33—0 45
Duration of administration (days)	3	3	8
Plasma cholesterol (mg/100 ml)			
Before exposure	262	203	279
Last day of exposure	156	158	200
Urate in 24-h urine (g per g creatinine mean and range)			
During exposure	2.22 (2.02—2.34)	1.23 (1.12—1 48)	0.72 (0.60—1.09)
Control periods (3—5 days)	1.24 (1.04—1.49)	0.94 (0 73—1.10)	0.51 (0.40—0.60)
17-OHCS in 24-h urine (mg per g creatinine mean and range)			
During exposure	28.7 (26.0—31.5)	21.6 (19 7—23.3)	8.7 (8 0—10 9)
Control periods (3—5 days)	17 4 (12.5—22.5)	17.2 (11.7—23.4)	7.8 (7.5— 10)

The daily dose of fructose was divided into 3—5 portions and given by mouth. Urine was collected in 24-h periods. Fasting blood samples were drawn every morning and analyzed for cholesterol, which in every case showed a gradual decline to the final value shown in the table. There was no consistent change in the FFA or TG concentration. Urinary protein content was also measured no rise was observed.

tinosis. It may cause renal calculi and potassium depletion. Proteinuria is a rather constant feature of HFI patients exposed to fructose. It has been reported to appear after one 50 g dose of fructose in an adult patient (46) In the experiments shown in Table 2 a prolonged exposure to fructose caused no rise in urinary protein

GENETICS

In most of the families reported, the oc currence of HFI conforms to autosomal recessive inheritance A few families are known (76 29 55) with an apparent autosomal dominant inheritance These may be instances of pseudodominance i e marriages between a homozygote and a heterozygote, but real genetic and biochemical heterogeneity of the syndrome is also possible. A test to reveal the heterozygous state for HFI would be of great value. We have measured liver F 1 P aldolase activities in 5 parents of HFI patients, but the results were not different from controls (48). Beyreiss et al have reported a higher blood fructose concentration plateau during constant fructose infusion in the parents of his HFI patient than in controls (2) This suggests decreased metabolic clearance of fructose in the heterozygous state, but the finding has not been confirmed in a larger series of heterozygotes.

DIAGNOSIS

Any infant presenting any of the clinical features of HFI, discussed above, should be placed on a fructose- free diet. A clear improvement of the condition in a few days suggests HFI. In older patients, the dietary history of aversion to sugar sugar-containing foods, fruits and berries is almost diagnostic. The diagnosis is confirmed by an intravenous fructose test. 0.25 g/kg is given as a 10 % solution in 2 min and blood glucose and serum inorganic phosphate are measured at 20 min intervals for 60 min. A distinct fall occurs in both in HFI. Recently a patient was reported with a defect of liver fructose diphosphatase activity (1) That patient was

indistinguishable from HFI patients on fructose test, but also had fasting hypoglycemia which is not a feature of HFI, and had no aversion to sweet. Two sisters reported by Dormandy and Porter (13) had severe hypoglycemia after both fructose and galactose, but also they had no distaste for sweet.

Another case is on the record (8) with a hypoglycemic response to fructose but with normal activities of F 1 P aldolase and fructose diphosphatase in the liver

TREATMENT

Many of the patients seem able to spontaneously select a fructose-free diet. However strict instructions are necessary (43) Recently glucose was recommended to be added to the diet, on the speculation that the liver fibrosis seen in the patients still after years on a fructose-free diet be due to lack of glucose (27). We are strongly against any trial to break the unique self protection that the patients have developed in their intense aversion to sweet taste, and prefer adding more starch to the diet if the theory turns out to be valid.

ACKNOWLEDGEMENT

This work was supported by the N tional Research Council for Medical Sciences, Finland

ADDENDUM

After the manuscript was finished studies have been performed in two mothers of HFI patients according to the protocol of Beyreiss et al (2) In contrast t their finding no difference from controls was observed (Perheentupa, J and Granstrom, M L., unpublished results)

REFERENCES

1 Baker L. & Winegrad, A. I Fasting hypoglycemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity Lancet II 13, 1970.

2. Beyreiss, K., Willgerodt, H. & Theile, H. Untersuchungen bei heterozygoten Merkmalsträgern für Fructoseintoleranz. Klin. Wochenschr 46 465, 1968.

3. Birke, G Diczfalusy E. & Plantin, L.-O Assessment of the functional capacity of the adrenal cortex. I. Establishment of normal values. J clin. Endocr 18 736, 1958.

4. Burch, H. B., Lowry O. H Meinhardt, L., Max, P J & Chyu, K. Effect of fructose, dihydroxyacetone, glycerol, and glucose on metabolites and related compounds in liver and kidney J biol. Chem. 245 2092, 1970.

5. Butenandt, I. Fructoseintoleranz. Verh. deutsch. Kongr inn. Med. 70·891, 1964.

6 Carlson, L. A. Determination of serum triglycerides. J Atheroscleroeis Res. 3 334, 1963.

7 Chaptal, J Jean, R., Bonnet, H Castel, J & Morel, G Vomissement cétonémiques *symptomatiques d'une intolérance congénitale* au fructose chez deux soeurs. Arch. franc. Pédiat. 25 745 1968.

8. Corbeel, L. M., Eggermont, E., Bettens, W Casteels-van Daele, M. & Timmermans, J.. Fructose intolerance with normal liver aldolase. Helv paediat. Acta 25 626, 1970.

9 Cornblath, M., Rosenthal, I. M., Reisner S. H., Wybregt, S. H. & Crane, R. K. Hereditary fructose intolerance. New Engl. J Med. 269 1271, 1963.

10. Crout, J R. Catechol amines in urine. Stand. Meth. Clin. Chem. 3 62, 1961.

11. Desbuquois, B.. L'intolerance hereditaire au fructose (déficit constitutionnel en 1 phosphofructaldolase). Rev int. Hépat. 15 1, 1965.

12. Desbuquois, B., Lardinois, R., Gentil, C. & Odievre, M. Effects d'une surcharge en phosphate de sodium sur l'hypoglucosemie. Dans onze observations d'intolerance hereditaire au fructose. Arch. franc. Pediat. 26 21 1969

13. Dormandy T L. & Porter R. J Familial fructose and galactose intolerance. Lancet 1 1189 1961

14. Dubois, P R., Loeb, H., Ooms, H. A., Gillet, P Bartman, J & Champenois, A. Etude d'un cas d'hypoglycémie fonctionnell par intolérance au fructose. Helv paediat. Acta 16 90, 1961.

15. Froesch, E. R. & Ginsberg, J Fructose metabolism f adipose tissue. I. Comparison f fructose and glucose metabolism in epididymal adipose tissue of normal rats. J biol. Chem. 237 3317 1962.

16. Froesch, E. R., Prader A., Wolf, H. P & Labhart, A. Die hereditäre Fructoseintoleranz. Helv paediat. Acta 14 99 1959

17 Froesch, E. R., Prader A., Labhart, A., Stuber H. W & Wolf H. P Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung. Schweiz. med. Wschr 87 1168 1957

18 Froesch, E. R., Wolf, H. P Baitsch, H., Prader A. & Labhart, A. Hereditary fructose intolerance. An inborn defect of hepatic fructose-1 phosphate splitting aldolase. Amer J Med. 34 151 1963.

19 Gentil, C., Colin, J Valette, A. M., Alagille, D. & Lelong, M. Etude du métabolisme glucidique au cours de l'I tolerance héréditaire au fructose. Essai d'interprétation de l'hypoglucosémie Rev Franc. et clin. biol. 9 596, 1964

20 Grant, D. B Alexander F W & Seakins, J W T Abnormal tyrosine metabolism in hereditary fructose intol rance. Acta Paediat. scand. 59 432, 1970.

21 Hers, H. G & Joassin, G Anomalie de l ldolase hépatique dans l'intolerance au fructose Enzymol. biol. clin. 1 4 1961

22. Higgins R. B & V rney J K. Dissolution f renal calculi in a case f hereditary fructose int lerance and renal tubular acidosis. J Urol. 95 291 1966.

23. Hlu, I. J Amiel, J L. & Mkdjou, K. Hormonal response to intravenous fructose in normal nd fructosaemic children a study of insulin and growth hormone secretion. Europ J clin. biol. Res 15 887 1970.

24 Jeun M Planson, E., Cotte J Bonnefoy S M elon, J L. & Skosowsky J L'intolérance héréditaire au fructose, à propos d'un Pediatrie 16 605, 1961

25 Kaplan. M Straus, P Brevart, P Gorouben, J-C & Bedu Saada, J Etude clinique et bi logique d un cas d'intolérance au fructose chez un nourisson Sem. Hôp. Paris 39 2733, 1973

26 F hlin, P & M lin, K Hereditary fructose intolerance in four Swedish families. Acta paediat scand 57 4 1968

27 Lelong, M Alagill D Le Tan Vinh, Gautier M & Odievr M Histologische Untersuchung der Leber bei der angeborenen Fructose-Intoleranz unter diätetischer Behandlung Mschr Kinderheilk 118 257 1970

28. Lindemann, R Gjessing, L. R Merton, B Loken, A C & Hal orsen, S Amino acid metabolism in hereditary fructosemia. Acta paediat. scand 9 141 1970

29 Lindén, L. & I uell. J Hereditä fruktosintolerans. Svenska Läk Tidn. 61 3187 1964.

30. Mäenpää, P H., Raivio, K. O & Kekomäki M. P Liver adenine nucleotides: Fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.

31 Mass R. E., Smith, W R. & Walsh, J R. The association of hereditary fructose intolerance and renal tubular acidosis. Amer. J med. Sci. 251 516 1966.

32. Métais, P., Juif J & Saerez, R.. Etude biochimique d'un cas d'intolérance héréditaire au fructose Ann. Biol. Clin. 20 801 1962

33. Morris, R. C. Jr An experimental renal acidification defect in patients with hereditary fructose intolerance. I. Its resemblance to renal tubular acidosis. J clin. Invest. 47 1389, 1968.

34. Morris, R. C. Jr An experimental renal acidification defect in patients with hereditary fructose intolerance II. Its distinction from classic renal tubular acidosis its resemblance to the renal acidification defect associated with the Fanconi syndrome of children with cystinosis. J clin. Invest. 47 1648 1968.

35 Morris, R. C. J Ueki, L., Loh, D Eanes R. Z. & McLin, P Absence of renal fructose-1-phosphate aldolase activity in hereditary fructose intolerance. N ture 214 920, 1967

36. Nikkilä, E. A. Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. Advanc. Lipid Res. 7 63 1969

37 Nikkilä, E. A. & Perheentupa, J Non-esterified fatty acids and fatty liver in hereditary fructose intolerance Lancet II 1280, 1962

38. Nikkilä, E. A., Somersalo, O Pitkänen, E & Perheentupa, J Hereditary fructose intolerance an inborn deficiency of liver aldolase complex. Metabolism 11 727 1962.

39. Nisell, J & Lindén, L. Fructose-1 phosphate aldolase and fructose 1,6-diphosphate aldolase in the mucosa of the intestine in hereditary fructose intolerance. Scand. J Gastroenterol 3 80, 1968

40. Nordmann, Y Schapira, F & Dreyfus, J C. A structurally modified liver aldolase in fructose intolerance Immunological and kinetic evidence Biochem. biophys. Res. Commun. 31 884, 1968.

41 Odievre M., Gautier M. & Rieu, D Intolérance héréditaire au fructose du nourrisson. Evolution des lésions histologiques hépatiques sous traitement diététique prolongé (Etude de huit observations). Arch. franc. Pediat. 26 433, 1969

42. Penhoet, E Rajkumar R. & Rutter W J Multiple forms of fructose diphosphate al dolase in mammalian tissues. Proc. nat. Acad. Sci. 56 1275, 1966.

43. Perheentupa, J & Hallman, N Hereditary fructose intolerance. In Endocrine and genetic disease of childhood. (ed. L. I. Gardner). p. 844. W B. Saunders Company Philadelphia 1969

44. Perheentupa, J Pitkänen, E Nikkilä, E. A., Somersalo, O & Hakosalo, J Hereditary fructose intolerance. A clinical study of four cases. Ann. paediat. fenn. 8 221 1962.

45. Perheentupa, J & Raivio, K Fructose-induced hyperuricaemia. Lancet 2 528, 1967

46. Phillips, M. J Little J A. & Ptak, T W Subcellular pathology of hereditary fructose intolerance. Amer J Med. 44 910, 1968

47 Raivio, K. O Kekomaki, M. P & Mäenpaa, P H. Depletion of liver adenine nucleotides induced by d-fructose Dose-dependence and specificity of the fructose effect Biochem. Pharmacol. 18 2615 1969

48. Raivio, K., Perheentupa, J & Nikkila, E A Liver aldolase activity in parents of children with hereditary fructose intolerance. Clin. chim. Acta 17 275, 1967

49 Raju, L., Chessels, J M & Kemball, M Manifestation of hereditary fructose intoler ance. Brit. med. J 2 446, 1971

50 Royer P Lestradet, H., Habib R., Lardinois, R. & Desbuquois, B. L'intolérance héréditaire au fructose. Bull. Mém. Soc. méd. Hôp. Paris 115 805, 1964.

51. Sacrez, R., Juif, J -G Metais, P Sofatzis, J & Dourof, N.. Un cas mortel d'intolérance héréditaire au fructose Etude chimique et enzymatique. Pediatrie 17 875, 1962.

52. Schapira, F & Dreyfus, J C. L aldolase hépa tique dans l'intolerance au fructose. Rev franç. Et. clin. biol. 12 486, 1967

53. Trout, D L., Estes, E. H., J & Friedberg, S. J Titration f free fatty acids of plasma. a study f current methods and a new modification. J Lipid Res. 1 199 1960.

54. Willems, C., Heusden, A., Renson, P Legat, C., Monard, Y & Stainier L. Hypertyrosinémie avec hyperméthioninémie néonatales dans un cas d'intolérance auf fructose. Helv paediat. Acta 26 467 1971

55. Wolf, H., Zschocke, D Wedemeyer F W & Hubner W Angeborene hereditäre Fructose-Intoleranz. Klin. Wschr 37 693, 1959

56. Woods, H. F Eggleston, L. V & Krebs, H. A. The cause of hepatic ccumulation of fructose 1-phosphat on fructose loading. Biochem. J 119 501 1970.

57 Zalitis, J & Oliver I. T Inhibition of glucose phosphate isomerase by metaboli intermediates of fructose. Biochem. J 102 700 1967

17 Froesch, E. R., Prader A., Labhart, A., Stuber H. W & Wolf, H. P Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung. Schweiz. med. Wschr 87 1168, 1957

18. Froesch, E. R., Wolf, H. P Baitsch, H., Prader A. & Labhart, A.. Hereditary fructose intolerance. An inborn defect of hepatic fructose-1 phosphate splitting aldolase Amer J Med. 34 151 1963.

19 Gentil, C., Colin, J Valette, A. M., Alagille, D & Lelong, M. Etude du métabolisme glucidique au cours de l'intolerance héréditaire au fructose Essai d'interprétation de l'hypoglucosémie. Rev Franc. et clin. biol. 9 896, 1964.

20. Grant, D. B., Alexander F W & Seakins, J W T Abnormal tyrosine metabolism in hereditary fructose intolerance Acta Paediat. scand. 59 432, 1970.

21 Hers, H. G & Joassin, G Anomalie de l aldolase hépatique dans l'intolerance au fructose Enzymol. biol. clin. 1 4, 1961

22. Higgins, R. B. & Varney J K. Dissolution of renal calculi in case of hereditary fructose intolerance and renal tubular acidosis. J Urol. 95 291 1966.

23. Hsu, I. J Amiel, J L. & Nikdjou, K.. Hormonal response to intravenous fructose in normal and fructosaemic children a study of insulin and growth hormone secretion. Europ. J clin. biol. Res 15 887 1970

4 Jeune M Plauson, E., Cotte, J Bonnefoy S Nivelon, J L. & Skosowsky J L'intolérance héréditaire au fructose, à propos d un cas Pediatrie 16 605 1961.

5 Kaplan, M Straus, P Brevart, P Gorouben, J-C & Bedu-Saada, J Etude clinique et biol g que d'un cas d'intolérance au fructose chez un nourisson. Sem. Hôp. Paris 39 2733, 1963

26. Kohlin, P & Melin, K. Hereditary fructose intolerance in four Swedish families. Acta paediat scand 57 4, 1968.

27 Lelong. J Alagill D Le Tan Vinh, Gautier M & Odievre M Histologische Untersuchung der Leber bei der angeborenen Fructose-Intoleranz unter diätetischer Behandlung. Mschr Kinderheilk. 118 257 1970

28. Lindemann, R Gjessing, L. R., Merton, B., Löken, A. C & Halvorsen, S. Amino acid metabolism in hered tary fructosemia. Acta paediat. scand 59 141, 1970.

29 Lindén, L. & Nisell, J Hereditär fruktosintolerans. Svenska Läk. Tidn. 61 3187 1964.

30. Mäenpää, P H., Raivio, K. O. & Kekomäki, M. P Liver adenine nucleotides Fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.

31 Mass, R. E., Smith, W R. & Walsh, J R. The association of hereditary fructose intolerance and renal tubular acidosis. Amer. J med. Sci. 251 516, 1966.

32. Métais, P Juif J & Sacrez, R. Etude biochimique d'un cas d'intolérance héréditaire au fructose. Ann. Biol. Clin. 20 801 1962.

33. Morris, R. C. Jr An experimental renal acidification defect in patients with hereditary fructose intolerance. I. Its resemblance to renal tubular acidosis. J clin. Invest. 47 1389 1968

34. Morris, R. C. Jr An experimental renal acidification defect in patients with hereditary fructose intolerance. II Its distinction from classic renal tubular acidosis its resemblance to the renal acidification defect associated with the Fanconi syndrome of children with cystinosis. J clin. Invest. 47 1648, 1968.

35 Morris, R. C. J Ueki, L., Loh, D Eanes, R. Z. & McLin, P Absence of renal fructose-1 phosphate aldolase activity in hereditary fructose intolerance. Nature 214 920 1967

36. Nikkilä, E. A. Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. Advanc. Lipid Res. 7 63, 1969

37 Nikkilä, E. A. & Perheentupa, J Non-esterified fatty cids and fatty liver in hereditary fructose intolerance. Lancet II 1280 1962

38. Nikkilä, E. A., Somersalo, O Pitkänen, E & Perheentupa, J Hereditary fructose intolerance, an inborn deficiency of liver aldolase complex. Metabolism 11 727 1962

39 Nisell, J & Lindén, L. Fructose-1 phosphate aldolase and fructose-1 6-diphosphate aldolase in the mucosa of the intestine in hereditary fructose intolerance. Scand J Gastroenterol. 3 80, 1968

40. Nordmann, Y Schapira, F & Dreyfus, J C. A structurally modified liver aldolase in fructose intolerance Immunological and kinetic evidence. Biochem. biophys. Res. Commun. 31 884 1968.

41. Odievre, M., Gautier M. & Rieu, D Intolérance héréditaire au fructose du nourrisson. Evolution des lésions histologiques hépatiques sous traitement diététique prolongé (Etude de huit observations). Arch. franc. Pediat. 26 433, 1969

Acta med. scand. Suppl. 542, pp. 77—83

HEREDITARY ALTERATIONS OF FRUCTOSE METABOLIZING ENZYMES

Studies on Essential Fructosuria and on Hereditary Fructose Intolerance

F Schapira, Y Nordmann and C. Gregori

Institut de Pathologie Moléculaire 24 rue du F bourg Saint J cques, Paris 14e — France

Abstract. Two hereditary alterations of fructose metabolizing enzymes are known. W have shown that in essential fructosuria, there is in fact deficiency of fructokinase activity W have shown that in hereditary fructose intolerance (HFI) some abnormal properties of aldolase in liver are related to aldolases A (muscle type) and C (brain type) which are normally synthesized by embryo, and which persist without change In livers with HFI, we have found protein immunologically related to aldolase B (liver dult type), the enzymatic activity of which is bout 3 per cent of the normal value. Its Michaelis constant f fructose-1 phosphate is greatly increased.

W conclude that, in hereditary fructose intolerance, there is mutation of th structural gene.

As shown by Hers (9) and then confirmed by Sillero et al. (25) the three enzymes of fructose metabolism in liver are fructokinase, liver type aldolase (aldolase B) and triosekinase. Heinz has reminded us of the normal metabolic pathway Two metabolic diseases, caused by a deficiency of enzymes of this metabolism are known (5) essential fructosuria (deficiency in fructokinase) and fructose intolerance (deficiency in aldolase B). We would like to study these two enzyme deficiencies here.

FRUCTOKINASE AND ESSENTIAL FRUCTOSURIA

Essential fructosuria was distinguished from hereditary fructose intolerance by Chambers et al. (1) and Froesch et al. (6). These last authors have put forward the hypothesis that the primary enzymatic defect is a lack of fructokinase. We have had the opportunity to confirm this hypothesis (24) and we shall recall briefly our findings, without describing the biological symptoms, or the genetic story· the diagnosis was made by a systematic investigation.

We used the method of Hers and Joassin (10) measuring the amount of fructose-^{14}C used up by fructokinase. Homogenates were incubated with fructose-^{14}C, ATP Mg^{++} and

Groupe U 15 d l'Institut N tional de la Santé et de la Recherche Médicale, Laboratoire associé au Centre N tional de la Santé et de la Recherche Médicale.

Table I. *Fructokinase and aldolase activities in patients with essential fructosuria and with hereditary fructose intolerance*

	Activities in μM of substrate utilized per minute and per gram			
		Aldolase		
	Fructokinase	FDP	F 1 P	FDP/F 1-P
Normal adult liver	0.05 to 0.12	6.0 to 14	6.0 to 14	1.0 to 1 1
Hereditary Fructose Intolerance (mean of biopsies, children and infants)	0 06	2.0	0.38	5.3
Essential Fructosuria	0	11.8	11.8	1.0

K^+ and after precipitation with barium, radioactivity of supernatant was estimated in reaction, and in blank without ATP

Under these conditions, normal adult liver used between 0.05 and 0 12 μM fructose per minute and per gram of wet liver In five livers from patients with fructose intolerance, we found the same values. On the other hand, in the liver of a subject with biological symptoms of essential fructosuria, the two aldolase activities were normal, and we found no fructokinase activity as seen in Table I.

The rarity of this anomaly (which is not a true disease) has not allowed us to perform other investigations on the enzymatic defect.

ALDOLASE AND HEREDITARY FRUCTOSE INTOLERANCE (HFI)

After the lecture of Perheentupa, we shall confine this chapter to some new data that we were able to bring up on the enzymic defect in HFI.

We confirmed, as other authors, the findings of Hers and Joassin (10) In the liver biopsies of 26 children with HFI we found a mean fructose-1 6-diphosphate (FDP) aldolase activity of 2.0 and fructose-1-phosphate (F 1 P) aldolase activity of 0.38 We point out that the colorimetric method that we used is more exact for the determination of F 1 P aldolase activity than the optical test (22). The mean aldolase activity ratio FDP F 1 P was 5 6 while it is normally between 1.0 and 1 1 As pointed out by Hers and ourselves, this high ratio is the characteristic enzymic anomaly in HFI. It is never encountered in other diseases, except in hepatoma as we have shown (13 20) We have shown earlier that several properties of embryonic liver were similar to properties of liver with HFI (19) We recall first that three types of aldolase are known (9 15 18) aldolase A (muscle type) aldolase B (liver type) and aldolase C (brain type) Table II summarizes the main properties of the three types in human tissues.

Spolter et al. (26) have shown that ATP competitively inhibits muscle-type aldolase, but not the liver type. We have found that

Table II. *Isoenzymes of aldolase in human tissues*

	Types		
	A	B	C
Activity ratio FDP/F 1 P	> 50	1.0	5 to 10
Electrophoretic migration (in starch gel) at pH 7 0	Anodic (slow)	Anodic (slow)	Anodic (fast)
Tissues (in which this type is preponderant)	Muscle	Liver	Brain

Table III. *Inhibition of FDP activity*

	Aldolase activity ratio FDP/F 1 P	Inhibition of FDP activity: Percentage of inhibition by ATP	Inhibition of FDP activity: Percentage of inhibition by antiserum antialdolase A
Human muscle	38	45	95
Human adult liver	1 0 t 1 1	< 12	< 15
Human fetal liver (2nd to 4th month)	2.0 t 3 0	25 to 35	30 to 45
Liver with HFI	5 6 (mean of 26 biopsies)	30 (3 experiments)	40 to 60

Concentration f ATP: 1 5 mM
Concentration of FDP 0 05 mM

Antiserum antialdolase A is prepared in chicken by repeated injections of crystalline rabbit muscl aldolase. This antiserum, like the antiserum antialdolase B, exhibits a good type specificity but a poor species specificity

aldolase of fetal liver and of liver with HFI is inhibited by ATP and also by the specific antiserum antialdolase A (muscle type). Table III summarizes our findings

We see that liver with HFI, like fetal liver contains muscle type aldolase. More recently our team has shown by kinetic, electrophoretic, and immunological methods, that fetal liver also contains brain type aldolase (aldolase C) (21 22). We are now able to characterize with electrophoresis, according to the method of Penhoet et al (15) the FDP aldolase activity in three livers with HFI. As shown in Fig 1, the pattern is similar to the brain pattern, and t seems that in HFI liver there are (as in fetal liver) hybrids of the two aldolases A and C.

Are the aldolases A and C really increased in liver with HFI? It seems to us that these embryonic forms are without change and only relatively increased. We recall that the specific activities of aldolases A and C are much greater than that of aldolase B an amount of 2 or 3 per cent may represent about 15 per cent of total FDP activity We have consequently proposed that the abnormal properties of aldolase in HFI are related to aldolase which is normally synthesized by

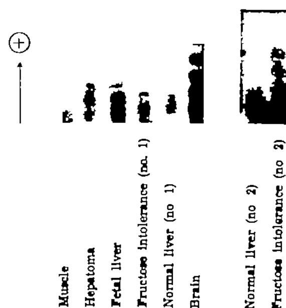


Fig 1. Aldolase isozymes of human tissues (substrate FDP). The concentration of liver HFI extract is much greater than the concentration of the other extracts.

Table IV *Percentage of protein cross reacting (CRM) with aldolase B in livers with fructose intolerance*

Human tissues	Proteins mg/0.1 ml	Removal of neutralizing antibody (units) (*)	Normal liver proteins mg/0 1 ml (**)	CRM percentage (***)
Fructose intolerance				
Liver a	0.75	0 66	0.18	24
b	0 80	0 71	0.24	30
c	1 16	0.24	0.21	18

(*) One unit Amount of antibody inhibiting 0.06 unit of FDP cleavage activity

(**) Amount of normal liver proteins producing an equivalent removal of antibody

(***) $\frac{\text{column 3}}{\text{column 1}} \times 100$

embryo and which persists without change. Aldolase B activity would be almost entirely lacking so that the aldolase A (and also the aldolase C) would become the main aldolase present in these livers.

But is aldolase B synthesized in livers with HFI? We brought evidence for the presence of a protein sharing the immunological properties of aldolase B in the liver of three patients (14 23)

Antiserum antialdolase B was prepared in chicken by repeated injections of pure aldolase B (according to the method of Morse and Horecker (12)). We have compared the removal of neutralizing antibodies by normal liver and by liver with HFI. After incubation, and centrifugation, the supernatant was assayed for residual antibody by determining the inhibition of FDP activity of pure aldolase B.

The amount of Cross Reacting Material (CRM) in the studied tissue was estimated by plotting our results on a standard curve where increasing amounts of liver proteins were incubated with 0 1 ml of antiserum anti aldolase B

Under these conditions, we have found in three livers respectively 4 18 and 30 per cent of the normal content of an immunological aldolase protein, as shown in Table IV

Comparing this amount of immunologically reactive protein and the F 1 P aldolase activity (which is about 2 to 3 per cent of the normal) we concluded that a Cross Reactive Material (CRM) does exist in these three patients.

Consequently we may propose that the synthesis of aldolase B persists, but that the enzyme is abnormal and almost without catalytic activity By the method of double immunodiffusion of Ouchterlony we were able to find a band of precipitation between the well with antiserum antialdolase B and the extracts of four different liver biopsies with HFI but these lines exhibited only a partial identity with the precipitation lines between antiserum anti B and extracts of normal liver

In order to confirm the anomaly of the hepatic aldolase we have estimated the Michaelis constant for its specific (or almost specific) substrate, fructose-1 phosphate because it must be recalled, indeed, that the main part of FDP activity is probably due to aldolases A and C.

Although the F 1 P aldolase activity is very low we have nevertheless succeeded in measuring the affinity of aldolase towards this substrate.

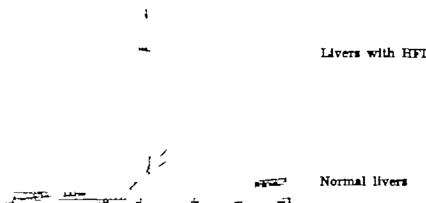


Fig. 2. K_m for the substrate fructose-1 phosphate in normal livers and livers with fructose intolerance. Abscissa (1/S) reciprocal of F 1 P concentration. Ordinate (1/V) reciprocal of percentage of aldolase activity

Fig. 2 shows the results which we obtained in normal adult liver in fetal liver (2—4 months) and in seven livers with HFI

It is seen that the K_m for F 1 P is between 2 mM and 3 mM in normal livers (adult or fetal) Gürtler et al. (6) found the same value after purification. On the contrary we find for these pathological livers, values from 10 to 60 mM. In two more recent biopsies we have found a K_m of about 30 mM.

Moreover we have recently found that if our extraction is performed with a buffer with β-mercaptoethanol and EDTA the affinity towards F 1 P becomes normal. On four biopsies where we measured the K_m, either in a water extract or in a β-ME buffer extract, we have found respectively a K_m between 15 and 20 mM in water and less than 3 mM in β-ME buffer with EDTA

On the contrary normal liver and brain (which exhibits a weak activity for F 1 P) show the same affinity for F 1 P whether they are extracted by water or by the β-ME buffer respectively 2 to 3 mM for liver and about 10 mM for brain.

We have found an abnormal affinity for F 1 P in the 10 biopsies which we were able to study On the other hand, we found a CRM only in three biopsies. In the three others, less than 10 per cent of CRM may be found. We have no explanation yet for this discrepancy It cannot be stated whether the protein is abnormally unstable or whether there is a true lack of synthesis of this protein in some cases.

In the latter case, we should have to conclude that there is a genetic polymorphism in cases with CRM, the mutation would be on the structural gene in other cases on a regulation gene. We recall the findings of Goedde and Altland (7) on the deficiency in serum pseudocholinesterase, where the percentage of protein immunologically related to the normal enzyme varied from 0 to 90 We recall also the analogous findings on the deficiencies in catalase (4) and in hypoxanthine guanine phosphoribosyl transferase (11) Nevertheless, our findings on the abnormal affinity in all cases are in favour of a structural mutation.

We should also like to discuss briefly the genetical problem. Raivio et al. (16) have studied liver biopsies from parents of patients with HFI, and have found an aldolase activity ratio close to the normal one. Nevertheless, we do not think that these interesting

---

W have verified by testing the action of antiserum anti-B that the F 1 P activity of livers with HFI is really due to aldolase B and not to aldolase C.

Table V *Calculation of aldolase activities in heterozygote (percentage of normal activity)*

	Aldolase activities		
	FDP	F 1 P	Ratio FDP/F 1 P
ALDOLASE B			
— synthesized by the normal gene	50	50	
— synthesized by the muted gene	1.5	1.5	
ALDOLASES A and C	10	0.5	
Total activity	61.5	51	1.2

results bring evidence for a mutation on a regulation gene with normal aldolase B in the heterozygote. In fact, the calculation shows that in heterozygote with a normal and an abnormal gene for aldolase B, the aldolase activity ratio could be no higher than about 1._ (Table V).

But the problem of normal FDP aldolase activity in parents of these patients as found by the authors is not solved.

CONCLUSIONS

It seems that in HFI there is a mutation of the structural gene Recently several examples of genetic diseases with synthesis of an abnormal protein without, or almost without, enzymatic activity have been described (1 3). Dreyfus has emphasized arguments in favour of structural mutations, and against mutations on a regulation gene in inherited diseases ( ) We believe that this concept is valid for HFI, and we hope that subsequent work will make it possible to determine more precisely the structural anomaly of aldolase in HFI, and also perhaps of fructokinase in essential fructosuria.

REFERENCES

1 Chambers, R. A. & Pratt, R. T C. Idiosyncrasy to fructose. Lancet 2 340, 1956.

2. Dreyfus, J C. The application of bacterial genetics to the study of human genetic abnormalities. In. Steinberg, A. G and Bearn, A. G Progress in Medical Genetics 6 10, 1969

3. Dreyfus, J C. & Alexandre, Y.. Immunological studies on glycogen storage diseases types III and V Demonstration of the presence of an immunoreactive protein in one case of muscle phosphorylase deficiency Biochem. biophys. Res. Comm. 44 1364, 1971.

4. Feinstein, R. N Suter H. & Jaroslow B. N Blood catalase polymorphism some immunological aspects. Science 159 638, 1968.

5 Froesch, E. R. Essential fructosuria and hereditary fructose intolerance In The Metabolic Basis of Inherited Diseases. Stanbury J B., Wyngaarden, J B. and Fredrickson, D S ed. McGraw Hill Book Co New York 124, 1966.

6. Froesch, E. R., Prader A., Labhart, A., Stuber, H. W & Wolf, H. P.. Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung. Schweiz. med. Wschr 87 1168, 1957

7 Goedde, H. W & Altland, K.. Evidence for different silent genes in the human serum pseudocholinesterase polymorphism. Ann. N Y Acad. Sci. 151 540, 1968.

8. Gürtler B Bally C. & Leuthardt, F.. Reindarstellung und Eigenschaften der menschlichen Leberaldolase. 19 Mitteilung über Aldolasen. Hoppe Seyler's Z. physiol. Chem. 352 1455, 1971

9 Hers, H. G Le Métabolisme du Fructose Arscia, Bruxelles 1957

10. Hers, H. G & Joassin, G Anomalie de l aldolase hépatique dans l'intolérance au fructose. Enzymol. biol. clin. 1 4, 1961.

11 Mc Donald, J A. & Kelly W N Lesch-Nyhan syndrome: altered kinetic properties of mutant enzymes. Science 171 689 1971.

12. Morse, D E. & Horecker B. L. Rabbit liver aldolase determination of the primary structure at the active site. Arch. Biochem. Biophys. 125 942, 1968.

13. Nordmann Y & Schapira, F Muscle type isoenzymes of liver aldolase in hepatomas. Europ J Cancer 3 247 1967

14 Nordmann, Y Schapira, F & Dreyfus, J. C. A structurally modified liver aldolase in fructose intolerance immunological and ki-

netic evidence. Biochem. biophys. Res. Comm. 31 884, 1968.

15. Penhoet, E., Rajkumar T & Rutter W J Multiple forms of fructose diphosphate aldolase in mammalian tissues. Proc. Nat. Acad. Sci. 56 1275 1966.

16. Raivio, K., Perheentupa, J & Nikkilä, E. A. Aldolase activities in the liver in parents of patients with hereditary fructose intolerance. Clin. chim. Acta 17 275 1967

17 Rubin, C. S., Dancis, J Yip, L. C., Nowinski, R. C. & Balis, M. E. Purification of IMP pyrophosphate phosphoribosyl transferases catalytically incompetent enzymes in Lesch Nyhan disease Proc. N t. Acad. Sci. 68 1461 1971

18. Schapira, F L'activité fructose-1 phospho-aldolasique des tissus de mammifères. I. Répartition de l'activité fructose-1 phospho-aldolasique dans les tissus de mammifères Bull. Soc. Chim. biol. 43 1357 1961

19 Schapira, F & Dreyfus, J C. L'aldolase hépatique dans l'intolérance au fructose. Rev franç. Et. clin. biol. 12 486 1967

20. Schapira, F Dreyfus, J C. & Schapira, G Anomaly of aldolase in primary liver cancer Nature 200 995 1963.

21. Schapira, F Hatzfeld, A. & Reuber M. D. Fetal pattern of aldolase in transplantable hepatomas. Cancer Res. 31 1224, 1971.

22. Schapira, F & Nordmann, Y.. Présence de trois types d'aldolase dans le foie humain. Clin. chim. Acta 26 189 1969

23. Schapira, F Nordmann, Y & Dreyfus, J C. La lésion biochimique de l'intolérance au fructose. Détection immunologique d'une aldolase modifiée. Rev franç. Et. clin. biol. 13 267 1968.

24. Schapira, F Schapira, G. & Dreyfus, J C., La lésion enzymatique de la fructosurie bénigne. Enzymol. biol. clin. 1 170, 1961/1962.

25 Sillero, M. A. G Sillero, A. & Sols, A. Enzymes involved in fructose metabolism in liver and the glyceraldehyde metabolic crossroads Europ. J Biochem. 10 345, 1969.

26. Spolter D Adelman, R. C. & Weinhouse, S. Distinctive properties of native and carboxypeptidase-treated aldolases of rabbit muscle and liver J biol. Chem. 240 1327 1965.

27 Tedesco, T A. & Mellman, W J Galactosemia evidence for a structural gene mutation. Science 172 727 1971



METABOLIC EFFECTS OF FRUCTOSE

Acta med. scand. Suppl. 542, pp. 87—103

HEPATIC ACCUMULATION OF METABOLITES AFTER FRUCTOSE LOADING

H. F Woods

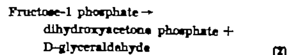
From the Department of the Regius Professor of Medicine and the Metabolic Research Laboratory N ffield Department f Clinical Medicine, Radcliffe Infirmary Oxf rd OX2 6HE, U.K.

Abstract. The changes in the metabolit content in freeze-clamped livers f fed rats occurring on perfusion with 10 mM D-fructose have been ex amined under aerobic and anaerobic conditions. During aerobic perfusion the main effects of fructose were an ccumulation of fructose 1 phosphate, as already known, up to 8.7 μmol/g of liver within 10 min, loss of total adenine nucleotides (up to 35 / after 40 min) with decrease in the ATP content to 23 / within 10 min, seven fold rise in the concentration of IMP to 1.1 μmol/g and an eight fold rise of α-glycerophosphate to 1.1 μmol/g. There was transient decrease in P from 4.2 to 1 7 μmol/g. Within 40 min the P content recovered to the normal value. The content f lactate increased to 4 3 μmol/g t 60 min pyruvate also increased and the [lactate]/[pyruvate] ratio remained within physiological limits. The concentration of free fructose within th liver remained much below that in the perfusion medium, indicating that the rate of penetration of fructose into the tissue was lower than the rate of utilisation. The fission of fructose 1 phosphate by liver aldolase is inhibited by several phosphorylated intermediates, especially by IMP This inhibition is competive with a K_i of 0.1 mM. The maximal rates of the enzyme synthesising and splitting fructose 1 phosphate are about equal. The ccumulation of fructose 1-phosphate on fructose loading is due to the inhibition of the fission of fructose 1-phosphate by the IMP arising from the degradation of the adenine nucleotides. When the conditions were anaerobi fructose was readily converted to lactate and the fructose 1-phosphate content of the liver after 40 min rose to 5.5 μmol/g. The total adenine nucleotide content decreased to 1.74 μmol/g. The contents of glycerophosphate and lactate were 4.3 μmol/g and 5.6 μmol/g respectively the [lactate]/[pyruvate] increased to 83.

D-fructose is metabolised in the mammalian liver mainly by the ketohexokinase (EC 2.7 1.3) reaction to fructose 1 phosphate (19 25)


Fructose + ATP → fructose-1-phosphate + ADP (1)

The further metabolism of fructose 1-phosphate is via the ketose-1-phosphate aldolase (EC 4.1.2.7) reaction.


Fructose-1 phosphate → dihydroxyacetone phosphate + D-glyceraldehyde (2)

Assays of these two enzymes in rat liver indicate that the rates of reaction are about the same. Fructose can be metabolised to

fructose 6-phosphate by the hexokinase (EC 2.7 1 1) reaction but the activity of this enzyme is low in liver (16) and it has a K_m of 2 4—6.7 mM for fructose (45) while that of ketohexokinase is 0.2—0.5 mM (1 31). In addition, the formation of fructose-6-phosphate via hexokinase is slow in the presence of glucose (47).

In 1942 Kjerulf-Jensen (22) found accumulation of fructose-1 phosphate in mammalian liver after a whole body fructose load (see also ref 9 15). Fructose is also rapidly metabolised by the isolated perfused rat liver under aerobic and anaerobic conditions (46 47) and this paper is concerned with the accumulation of hepatic metabolites, in addition to F 1 P after addition of D-fructose to the perfusion medium.

MATERIALS AND METHODS

Animals and diets Female Wistar rats each weighing 170—215 g obtained from Carworth (Europe) Ltd, Alconbury Hunts, U K., were used and were fed on a standard small-animal diet (Spillers Mills Ltd, Gainsborough, Lincs, U K.). Water was provided ad libitum.

Liver perfusion. The method of liver perfusion used was that described by Hems et al. (18). The perfusion medium consisted of physiological saline (24) with 2.5 bovine serum albumin fraction (Armour Pharmaceutical Co. Ltd, Eastbourne Sussex, U K.), and aged human erythrocytes, which were thoroughly washed as described by Woods et al (46) to lower their initial lactate content The bovine serum albumin was dialysed as a 10 % solution (at 4 C) against three changes of physiological saline gassed with CO_2 + O_2 (5 95). In anaerobic experiments where O was to be excluded from the perfusion apparatus and medium the conditions of perfusion were altered as previously described (4), i e the washed erythrocytes were omitted from the medium and the perfusions were carried out in the presence of cyanide which was added as an 0.2 M solution of KCN neutralised with HCl to give final concentration of 1 mM in the medium The medium was gassed with CO_2 + N_2 (5 95)

For other methodological details see Woods et al. (46) and Woods and Krebs (47)

RESULTS

Content of intermediary metabolites in the freeze-clamped perfused rat liver after fructose loading

The main changes in the contents of liver metabolites after addition of 10 mM fructose to the perfusion medium were the accumulation of fructose-1 phosphate and a loss of hepatic adenine nucleotides as described by Mäenpää et al. (27) and Raivio et al. (33) (Table I).

The concentration of ATP decreased to 23 % within 10 min and then slowly increased, reaching 48 % of the original value at 80 min. The sum of the adenine nucleotides decreased to 42 % in 10 min and was 50 % at 80 min. Some of the lost adenine nucleotides appeared as IMP the concentration of which increased seven-fold within 10 min and then decreased, but the lost adenine nucleotides were not fully accounted for by the substances analysed. This deficit increased with time. The findings of Mäenpää et al. (27) (made under slightly different conditions) suggest that the adenine nucleotides were partly broken down to uric acid and allantoin.

Among the phosphorylated products fructose 1 phosphate increased 40-fold within 10 min to 8.7 µmol/g and α-glycerophosphate rose eight fold to 1 06 µmol/g Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate increased about four fold. The changes in the concentrations of other phosphorylated compounds, though substantial on a percentage basis in the case of fructose 1 6-diphosphate glucose-6-phosphate phosphoenol pyruvate 2 phosphoglycerate and 3-phosphoglycerate were small in absolute terms.

There was a large decrease in the concentration of P at 10 min. The value of P_i had returned to normal at 40 min and was increased above normal at 80 min. The total phosphorus content of the liver calculated

Table I. *Contents of intermediary metabolites in the perfused rat liver*

Livers of fed rats were perfused with a medium containing D-fructose (initial concentration 10 mM) and were freeze-clamped t various times after the start of the perfusion. The initial values refer to livers of fed rats freeze-clamped *in vivo* after cervical dislocation. The results are means ± S.E.M.

	Livers not perfused	Livers perfused with D-fructose		
Time of perfusion (min)	0	10	40	80
Number of observations	4	4	4	3
Content of metabolites (μmol/g wet wt.)				
Fructose-1-phosphate	0.23 (2)	8.72 ± 0.83	7.68 ± 0.98	3.27 ± 1.31
ATP	2.22 ± 0.07	0.51 ± 0.16	0.77 ± 0.11	0.99 ± 0.01
ADP	0.78 ± 0.08	0.66 ± 0.07	0.20 ± 0.02	0.50 ± 0.10
AMP	0.26 ± 0.05	0.20 ± 0.03	0.17 ± 0.02	0.14 ± 0.003
Total adenine nucleotides	3.27 ± 0.12	1.37 ± 0.08	1.14 ± 0.13	1.84 ± 0.13
P	4.25 ± 0.25	1.67 ± 0.29	2.97 ± 0.26	5.62 ± 0.28
IMP	0.12 ± 0.03	1.14 ± 0.08	0.37 ± 0.13 (3)	0.26 ± 0.02
α-Glycerophosphate	0.13 ± 0.10	1.06 ± 0.23	0.60 ± 0.08	0.45 ± 0.07
D-Glyceraldehyde	—	—	0.10 (2)	—
Free fructose	< 0.01	3.86 ± 0.51	0.82 ± 0.20	0.22 ± 0.11
Glucose	7.22 ± 0.77	2.29 ± 0.65	6.01 ± 0.45	5.91 ± 0.16
Lactate	0.79 ± 0.09	1.34 ± 0.15	3.57 ± 0.34	4.22 ± 0.68
Pyruvate	0.08 ± 0.01	0.15 ± 0.03	0.35 ± 0.02	0.36 ± 0.06
Fructose-1,6-diphosphate	0.02 ± 0.002	0.10 ± 0.02	0.03 ± 0.01	0.02 ± 0.003
Glucose-6-phosphate	0.25 ± 0.03	0.14 ± 0.04	0.38 ± 0.04	0.20 ± 0.08
Fructose-6-phosphate	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.07 ± 0.02
Glucose-1-phosphate	< 0.01	< 0.01	0.01 ± 0.001	< 0.01
Phosphoenolpyruvate	0.16 ± 0.02	0.05 ± 0.02	0.19 ± 0.03	0.22 ± 0.01
2 Phosphoglycerate	0.04 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.08 ± 0.003
3-Physphoglycerate	0.26 ± 0.06	0.16 ± 0.03	0.47 ± 0.06	0.57 ± 0.06
Glyceraldehyde-3-phosphat	0.006 ± 0.0002	0.03 ± 0.004	0.006 ± 0.0003	0.007 (2)
Dihydroxyacetonephosphate	0.04 ± 0.003	0.16 ± 0.04	0.06 ± 0.002	0.06 (2)
[Lactate]/[pyruvate]	9.60 ± 0.96	10.1 ± 1.22	10.4 ± 1.24	12.0 ± 0.73
[Fructose-6-phosphate]/ [glucose-6-phosphate]	0.25 ± 0.03	0.57 ± 0.12	0.30 ± 0.01	0.35 ± 0.06
[Glucose-6-phosphate]/ [glucose-1-phosphate]	—	—	27.5 ± 3.8	—
[2 Phosphoglycerate]/ [3-phosphoglycerat]	0.20 ± 0.06	0.19 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
[-Glycerophosphate]/ [dihydroxyacetone phosphate]	3.65 ± 0.40	9.28 ± 3.62	9.61 ± 1.08	8.53 ± 1.95
[ATP] [AMP]/[ADP]²	0.97 ± 0.17	0.31 ± 0.17	3.50 ± 0.70	0.90 ± 0.17

from the mean concentration of phosphorylated intermediates, adenine nucleotides, IMP and P was increased at 10 min and at 40 min on perfusion with D-fructose. Livers freeze-clamped *in vivo* contained 13.8 μmol of total phosphorus/g whereas those perfused with fructose contained 16.3 μmol/g at 10 min and 40 min. The total phosphorus content returned to normal (14.3 μmol/g) at 80 min. The transient increase in total phosphorus content was due to an increase in the concentration of phosphorylated intermediates. The values for total phosphorus content are approximate because a few intermediates such as UTP and UDP-glucose that decrease in concentration during treatment with D-fructose (9) were not determined in the freeze-clamped livers. However the contribution of these intermediates is small. At 10 min and 40 min the increase in total phosphorus content represents an uptake of 19.68 μmol of phosphorus from the perfusion medium by 8 g of liver. This hepatic phosphorus uptake may account for the transient

Table II. *Balance of fructose metabolism in the perfused rat liver*

The amounts of products formed represent the sum of metabolites in medium and liver The quantities of most other intermediates in medium and liver (listed in Table I) were negligibly small and have therefore been omitted from the calculation of the balance.

Expt. no.	Liver wt. (g)	Duration of perfusion (min)	Fructose removed (µmol)	Fructose-1-phosphate (µmol)	Products formed: Lactate (µmol)	Pyruvate (µmol)	Glucose (µmol)	Sum of products (expressed in C_6 units) (µmol)
1	9.36	40	1048	62	894	30	701	1075
2	7.51	40	756	79	456	45	659	888
3	8.00	40	1065	89	707	40	749	1182
4	10.80	40	1049	66	696	87	702	1180
5	10.14	80	1328	27	1419	82	898	1680
6	9.20	80	1175	54	748	78	930	1397
7	9.95	80	1074	14	845	67	907	1227

lowering of serum P_i in human subjects during intravenous D-fructose infusion (26)

The concentrations of lactate and pyruvate increased steadily and in parallel. Lactate reached an average concentration of 4.3 µmol/g after 80 min which is much higher than the concentrations obtained in the absence of fructose when, under the same conditions, mM was rarely exceeded. The [lactate]/[pyruvate] ratio remained fairly constant throughout the perfusion period at a value near 10

The metabolite ratios were all of the order expected for equilibrium with the exception of the mass action ratio of the adenylate kinase system (ATP) (AMP)/(ADP)^2 which was outside the expected range after 10 and 40 min. Adenylate kinase is taken to be located in the outer mitochondrial membrane and to act slowly upon the cytoplasmic adenine nucleotides (4 11) As ketohexokinase AMP deaminase and 5-nucleotidase are cytoplasmic enzymes, the depletion of the adenine nucleotides after fructose loading is expected to concern the cytoplasm in the first instance If the adenine nucleotides that remain after fructose loading were mainly mitochondrial, some deviations of the mass action ratio from equilibrium would be understandable

Balance between fructose disappearance and metabolites formed

The sum of the products formed from fructose as calculated in Table II, was somewhat greater (up to 10 % at 40 min and up to 23 % at 80 min) than the amounts of fructose removed. This surplus is explained by the inclusion of some glucose formed from glycogen. The calculations indicate that the greater part of the fructose removed was converted into glucose It is unlikely that

Table III. *Rates of metabolite changes in the medium of the perfused rat liver*

The initial rates of metabolite changes during perfusion with D-fructose (initial concentration 10mM) were calculated from a plot of each metabolite in the total perfusion medium versus time. The results are means ± S.E.M. for seven observations. The experiments were the same as those in T ble II.

Metabolite changes	Rate (µmol/min per g wet wt)
Fructose removed	2.63 ± 0.23
Glucose formed	1.67 ± 0.22
Lactat formed	1 74 ± 0 16
Pyruvate formed	0 15 ± 0.01

there were significant quantities of metabolites other than those listed in Table I, and the complete oxidation of fructose could at most have contributed 10 / to the removal of fructose because the rate of oxygen consumption was 2.6 μmol/min per g of liver This could have oxidised 0 43 μmol of fructose/min whereas the rate of removal was 2.63 μmol/min per g of liver

The values for the main initial rates of fructose removal and glucose lactate and pyruvate production in the perfusion medium (Table III) were obtained for the balance experiments of Table II from a plot of metabolites in the total medium versus time. Linearity usually extended for about 60 min. The values for the rates confirm that virtually all the fructose removed during the earlier period of perfusion is accounted for by the formation of glucose, lactate and pyruvate

A comparison of the fructose content in the freeze-clamped perfused liver with that in the perfusion medium at the time of clamping shows that a gradient existed for this metabolite between the medium and the liver (Table IV). The concentrations in the liver were several fold lower The values for fructose content of the freeze-clamped liver have been corrected for the fructose content and volume of the entrapped medium by using the value 22.2 / obtained by Krebs et al. (23) for the extracellular space, which consists mainly of the liver vascular system and its contents.

The initial rate of fructose removal was 2.63 μmol/min per g wet wt. of liver and the existence of the concentration gradient implies that the rate of penetration of fructose is less than the rate of its metabolism.

Cause of accumulation of fructose 1 phosphate

The results outlined above can serve as a basis for the calculation of the rates of fruc

Table IV *Fructose content of liver and perfusion medium*

The values for fructose content of the liver have been corrected for the fructose content of the extracellular space using the figure f 22.2 / (23) The bulk of the extracellular space was the perfusion medium within the hepatic vascular system.

		Fructose content			
			Perfused liver		
Time (min)	Expt. no	Perfusion medium (μmol/ml)	Uncorrected (μmol/g wet wt.)	Corrected for fructose content and volume of the extracellular space (μmol/g wet wt.)	Fructose in medium/ Fructose in tissue (corrected) ratio
10	1	8.53	8.23	4.29	2 0
	2	8 06	4 04	2 89	2 8
	3	7 33	3.05	1 83	4 0
	4	8.74	3.12	1.53	5 7
40	1	2.60	0.56	<0 01	
	2	3 05	0.82	0.18	16.9
	3	4 52	1.39	0.50	9 0
	4	2.73	0 53	<0 01	
60	1	1.80	0.36	<0.01	
	2	0 23	0.01	<0 01	
	3	1.13	0 29	<0.01	

Table II. *Balance of fructose metabolism in the perfused rat liver*

The amounts of products formed represent the sum of metabolites in medium and liver The quantities of most other intermediates in medium and liver (listed in Table I) were negligibly small and have therefore been omitted from the calculation of the balance.

				Products formed				
Expt. no.	Liver wt. (g)	Duration of perfusion (min)	Fructose removed (μmol)	Fructose-1-phosphate (μmol)	Lactate (μmol)	Pyruvate (μmol)	Glucose (μmol)	Sum of products (expressed in C_6 units) (μmol)
1	9.36	40	1048	62	394	30	701	1075
2	7.51	40	786	79	456	45	659	888
3	8.00	40	1065	89	707	40	749	1182
4	10.80	40	1049	66	696	87	702	1160
5	10.14	80	1328	27	1419	52	896	1660
6	9.30	80	1175	54	748	78	930	1397
7	9.95	80	1074	14	845	67	907	1227

lowering of serum P in human subjects during intravenous D-fructose infusion (28)

The concentrations of lactate and pyruvate increased steadily and in parallel. Lactate reached an average concentration of 4.2 μmol/g after 80 min which is much higher than the concentrations obtained in the absence of fructose when, under the same conditions, 2 mM was rarely exceeded. The [lactate]/[pyruvate] ratio remained fairly constant throughout the perfusion period at a value near 10

The metabolite ratios were all of the order expected for equilibrium with the exception of the mass action ratio of the adenylate kinase system, (ATP) (AMP)/(ADP)^2 which was outside the expected range after 10 and 40 min. Adenylate kinase is taken to be located in the outer mitochondrial membrane and to act slowly upon the cytoplasmic adenine nucleotides (4 11) As ketohexokinase AMP deaminase and 5-nucleotidase are cytoplasmic enzymes, the depletion of the adenine nucleotides after fructose loading is expected to concern the cytoplasm in the first instance. If the adenine nucleotides that remain after fructose loading were mainly mitochondrial, some deviations of the mass action ratio from equilibrium would be understandable

Balance between fructose disappearance and metabolites formed

The sum of the products formed from fructose, as calculated in Table II, was somewhat greater (up to 10 % at 40 min and up to 23 % at 80 min) than the amounts of fructose removed. This surplus is explained by the inclusion of some glucose formed from glycogen. The calculations indicate that the greater part of the fructose removed was converted into glucose It is unlikely that

Table III. *Rates of metabolite changes in the medium of the perfused rat liver*

The initial rates of metabolite changes during perfusion with D-fructose (initial concentration 10mM) were calculated from a plot of each metabolite in the total perfusion medium versus time. The results are means ± S.E.M. for seven observations. The experiments were the same as those in Table II.

Metabolite changes	Rate (μmol/min per g wet wt)
Fructose removed	2.63 ± 0.28
Glucose formed	1.87 ± 0.22
Lactate formed	1.74 ± 0.16
Pyruvate formed	0.15 ± 0.01

Table V *Inhibitions by cell constituents of the rat liver aldolase reactions in tris hydrochloric acid buffer at pH 7 4*

The preparation of the liver extract and details of the assay are given in ref. 46 The effect of Mg^{2+} was tested when it was found that Mg^{2+} can bolish the inhibition of activity on fructose-1-phosphate by ATP The substances marked with an asterisk contained, according to the makers, between 1 and 6 / of the mono- and di-phosphates.

Substances tested (1 mM)	Percentage inhibition of aldolase reactions with Fructose-1-phosphate (0.067 mM)	Fructose-1 phosphate (0 067 mM) MgCl (7.5 mM)	Fructose-1,6-diphosphate (0 033 mM)
ATP	63	0	0
ADP	42	17	0
AMP	84	44	0
ITP	68	36	0
IDP	86	71	0
IMP	90	74	0
GTP	82	37	0
GDP	75	52	0
GMP	80	57	0
UTP	58	13	0
UDP	69	38	0
UMP	67	60	0
CTP	78	0	0
CDP	61	14	0
CMP	57	37	0
L-α-Glycerophosphate	35	37	20
2 Phosphoglycerate	44	37	6
3-Phosphoglycerate	41	38	0
Phosphoenolpyruvate	31	36	36
Glucose-1-phosphate	78	30	0
Glucose-6-phosphate	32	27	15
Fructose-6-phosphate	22	30	18
P	29	35	0
D-Glyceraldehyde	49	49	0

gave values ranging from 0 14 to 0.66 (average 0.35 mM fructose-1 phosphate) This is lower than the K_m values found for rabbit liver aldolase 0.8 mM (37) and 2 mM (43).

The effect of some intermediates and in hibitors on the aden ne nucleotide depletion and fructose 1 phosphate accumulation after fructose loading

The effect of the addition of adenine, aspartate, P and dipyramidole (a pyrimido-pyrimidine compound) on the adenine nucleotide depletion induced by fructose was investigated in perfused livers (Table VII). The hepatic contents of adenine nucleotides,

Table VI. *Effect of substrate and inhibitor concentration of the inhibition by IMP of rat liver aldolase fission of fructose 1 phosphate*

The preparation of the liver extract and method of the assay are given by Woods et al. (46). Corrections have been made for blank without fructose 1-phosphate.

Concentration of fructose-1-phosphate	Rate of fission of fructose-1-phosphate ($10^3 \times \Delta E$ in 6 min t 25 C) Control (no IMP)	0.5 mM IMP	1 0 mM IMP
0 25	118	30	17
0.5	145	53	27
1.0	163	82	52
2.5	180	115	89
5.0	186	142	121
10.0	180	153	139

tose-1 phosphate formed by the tissue compared with that accumulated. Within 10 min 8 7 μmol of fructose-1 phosphate had accumulated per g of tissue. Since the capacity of hexokinase is very low in relation to that of ketohexokinase (16) most of the other products formed from fructose (at a collective rate of 2.63 μmol/min per g, Table III) must have passed through the stage of fructose-1 phosphate about 30 % of the fructose-1-phosphate formed during the first 10 min had accumulated, and although the rate of fructose removal remained constant for another 35—50 min the concentration of fructose-1-phosphate did not continue to increase. This fact, together with the observation that the potential capacity of the aldolase is high enough to split more than 2.6 mol of fructose-1 phosphate/min per g (Table XIII) suggests that the accumulation of fructose-1 phosphate is caused by an inhibition of the aldolase that is overcome when the concentration of fructose-1 phosphate rises. The following experiments bear out this hypothesis.

A systematic search for inhibitors of the aldolase reaction among liver constituents (Table V) shows that several phosphorylated metabolites inhibit liver aldolase and that the fission of fructose-1 phosphate is much more sensitive to inhibitions than the fission of fructose-1 6-disposphate. However the concentration of the majority of potential inhibitors is not sufficiently high in the liver to be effective Possible exceptions are IMP AMP GMP UMP CMP and α-glycerophosphate. The tri- and di-phosphates of adenosine, guanosine uridine and cytidine are powerful inhibitors in the absence of Mg^{2+} but since these substances are present in the tissue mainly as Mg^{2+} chelates they cannot be major inhibitors *in vivo* The concentrations of GMP UMP and CMP are probably too low for them to be major inhibitors so that IMP and α-glycerophosphate must be taken to be the main agents that affect the activity of aldolase because the sum of the concentrations of GTP GDP GMP UTP UDP UMP CTP CDP CMP is less than 1.5 μmol/g of liver (6 20, 38) Adenosine, inosine, adenine, hypoxanthine, fructose and glucose were not inhibitory

The extent of the inhibition of fructose-1-phosphate fission by a particular inhibitor depends on the presence of other inhibitory phosphorylated compounds in the incubation medium. In general the inhibitions recorded in Table V were not additive. Measurements of the rate of fission in solutions designed to imitate the essential metabolite concentrations of the liver (as recorded in Table I) show that the inhibition in a liver environment equivalent to perfusion with 10 mM fructose for 10 min is 62 % and that equivalent to 40 min is 22 % provided that the fructose-1 phosphate concentration is at the normal value of 0.2 μmol/g. Increasing the fructose-1-phosphate concentration to 5 μmol/g decreased these inhibitions to 44 % and 16 % respectively at the two times. The main inhibitor under these conditions is IMP Although P_i alone is inhibitory this inhibition is not additive to that by IMP In further experiments not recorded in the Tables combinations of the four purine and pyrimidine mono- di and tri phosphates at physiological concentrations had no significant effects on the inhibition of fructose-1-phosphate fission by IMP and α-glycerophosphate.

Detailed information on the degree of inhibition of fructose-1 phosphate fission by IMP at different substrate and inhibitor concentrations is given in Table VI. A plot of 1/v against 1/s (26) from the data in Table VI indicates a competitive nature of the inhibition and a plot of 1/v against I according to Dixon (13) from this and other experiments gave an average K_i of 0.1 mM—IMP The K_m for the substrate from results in Table VI (graphical methods of Schwimmer (39) and Dixon (13)) was approximately 0 18 mM—fructose-1 phosphate but other experiments

Dipyramidole inhibits cardiac muscle adenosine deaminase (EC 3.5.4.4) *in vivo* (7). The effect of this compound on adenine nucleotide depletion was investigated by perfusing livers with a medium which contained 10 mM D-fructose and 0.1 mM dipyramidole. It was noted that these livers were the distinct yellow colour of dipyramidole when freeze-clamped which suggested that the drug was concentrated by the liver. The total adenine nucleotide content fell to 60 % of that *in vivo* at 10 and 40 min, thus adenosine deaminase may play a part in the adenine nucleotide depletion. In a separate experiment it was found that dipyramidole at a concentration of 0.1 mM did not inhibit rat liver aldolase reactions.

Hepatic metabolite contents during anaerobic perfusion in the presence of fructose

Rat liver slices are known to convert added fructose readily into lactate when the conditions are anaerobic if the rat has been well fed (12, 34, 35, 36). The rates of glycolysis in isolated perfused livers from well fed rats are much greater than those measured in tissue slices, and are increased by the addition of D-fructose, the anaerobic changes with regard to carbohydrate metabolism being the sum of the metabolism of endogenous glycogen and the added fructose (47). The changes in glycolytic metabolites in rat liver after ischaemia in situ have been investigated by Hems and Brosnan (17). Under the conditions of their experiments rates of glycolysis during the first two minutes after severance of the circulation were about half of those observed in the perfused organ (5).

In the experiments reported below the contents of adenine nucleotides, IMP and the intermediates of glycolysis were determined in freeze-clamped livers perfused under anaerobic conditions when the rates of glycolysis are rapid and comparable to those observed in tissues which have a high glycolytic capacity (brain, embryonic tissue, testicle, leucocytes, tumours). The intermediates were determined during anaerobic perfusion when the substrate for glycolysis was hepatic glycogen and during perfusion with a medium containing 10 mM D-fructose. The livers were freeze-clamped after 40 min of perfusion had elapsed when the time-course of glycolysis was linear.

Intermediates of glycolysis in livers perfused in the absence of added substrate. The contents of liver metabolites after 40 min of anaerobic perfusion are shown in Table VIII. Comparison with the values found in livers from fed rats freeze-clamped *in vivo* after cervical dislocation shows the following.

Adenine nucleotides, inorganic phosphate and IMP. The content of ATP decreased to 19 % of that found *in vivo*. The ADP also decreased to 67 % of the *in vivo* level. The fall in ATP and ADP content was accompanied by a two-fold rise in the AMP content. The total adenine nucleotide content fell to 40 % of control mainly due to the fall in ATP. The P content did not change significantly. The mean total phosphorus content of the liver calculated from the values obtained for the content of the phosphorylated intermediates, adenine nucleotides, IMP and P determined in each liver was 16.8 µmol/g wet wt., which is higher than that calculated for livers of fed rats freeze-clamped *in vivo* (13.8 µmol/g wet wt.). The calculated total liver phosphorus content was only approximate because all phosphorylated intermediates were not determined, and under anaerobic conditions, nucleotides other than the adenine nucleotides may have fallen in content. *Lactate and pyruvate.* There was a five-fold rise in the lactate content of the anaerobic liver. The pyruvate content fell by half and the [lactate]/[pyruvate] ratio rose from

Table VIII. *Contents of intermediary metabolites in the anaerobic perfused rat liver*

Livers from well fed rats were freeze-clamped after 40 min of perfusion under anaerobic conditions in the presence of 1 mM HCN The data refers to livers perfused without added substrate and to livers perfused in the presence of D-fructose (initial concentration 10 mM). The results are compared with those obtained for livers of well fed animals freeze-clamped *in vivo* following cervical dislocation. The metabolite contents are mean ± S.E.M.

	Metabolite content (μmol/g wet wt.)		
	Liver not *perfused*	Livers perfused with no added *substrate*	Livers perfused with D-fructose
Time of perfusion (min)	0	40	40
No. of observations	4	5	5
ATP	2.22 ± 0.07	0.43 ± 0.02	0.54 ± 0.02
ADP	0.79 ± 0.08	0.53 ± 0.08	0.71 ± 0.04
AMP	0.26 ± 0.05	0.55 ± 0.04	0.49 ± 0.02
Total adenine nucleotides	3.27 ± 0.12	1.51 ± 0.10	1.74 ± 0.05
IMP	0.12 ± 0.03	0.22 (2)	0.52 ± 0.04 (4)
Pi	4.25 ± 0.25	3.92 ± 0.46	4.44 ± 0.47
D-Glyceraldehyde	—	—	0.71 (2)
α-Glycerophosphate	0.13 ± 0.10	9.26 ± 0.56	4.30 ± 0.43
Fructose-1-phosphate	0.23 (2)	—	5.54 ± 0.78
Free fructose	< 0.01	—	2.68 ± 0.20
Free glucose	7.22 ± 0.77	9.48 (2)	7.15 ± 1.73
Lactate	0.79 ± 0.09	4.61 ± 0.74	5.62 ± 0.43
Pyruvate	0.08 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
Fructose-1,6-diphosphate	0.02 ± 0.002	0.03 ± 0.003	0.03 ± 0.005
Glucose-6-phosphat	0.25 ± 0.03	0.32 ± 0.05	0.31 ± 0.06
Fructose-6-phosphate	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.03
Glucose-1-phosphate	< 0.01	< 0.01	< 0.01
Phosphoenolpyruvate	0.16 ± 0.02	0.04 ± 0.003	0.08 ± 0.01
2 Phosphoglycerate	0.04 ± 0.01	0.03 ± 0.005	0.05 ± 0.01
3-Phosphoglycerate	0.26 ± 0.06	0.10 ± 0.01	0.16 ± 0.02
Glyceraldehyde-3-phosphate	0.006 ± 0.0002	0.01 ± 0.0007	0.012 ± 0.0025
Dihydroxyacetone phosphate	0.04 ± 0.003	0.12 ± 0.006	0.16 ± 0.021
Citrate	0.45 ± 0.06	0.09 (2)	0.19 ± 0.02
α-Oxoglutarate	0.11 ± 0.003	0.03 (2)	—

a value of 9.6 in fed livers freeze-clamped *in vivo to 101 in livers perfused under an* aerobic conditions.

Hexose phosphates Among the hexose phosphates the contents of FDP G-6-P and F-6-P all rose but the changes were small.

3-Carbon compounds The triose phosphates both rose, dihydroxyacetone phosphate threefold, while glyceraldehyde-3-phosphate almost doubled. The contents of phosphoenol pyruvate 2 PGA and 3-PGA all fell. The most striking change was the accumulation of α-glycerophosphate which rose seventy fold to 9.26 μmol/g wet wt.

These changes can be summarised by stating that above the triose phosphate level the intermediates of the glycolytic sequence all rose in content and those below fell.

Other intermediates. The glucose content rose by about one-third to 9.48 μmol/g wet wt. and the contents of the TCA cycle intermediates citrate and α-oxoglutarate both fell.

Metabolite ratios The metabolite ratios for some of the reactions of glycolysis calculated from the measured contents of intermediates in livers freeze-clamped *in vivo* and after 40 min of anaerobic perfusion (Table IX) indicate that the reactions catalysed by phos-

Table IX. *Metabolite ratios in perfused rat liver*

The metabolite ratios have been calculated from the contents in Table VIII for livers from well fed rats freeze-clamped *in vivo* and after 40 min of perfusion under anaerobic conditions with no added substrate and with D-fructose (initial concentration 10 mM). The ratios have been calculated in the direction of lactate formation except for the triose phosphate isomerase reaction. The adenylate kinase equilibrium has been calculated in the direction of ATP formation. Apparent equilibrium constants quoted were taken from the following references (10[1] 33[2] 44[3]).

Measured ratio	Fed liver *in vivo* (4)	Livers perfused under anaerobic conditions: No added substrate (5)	D-fructose (10 mM) (5)	Apparent Equilibrium Constant K'	Enzyme
[Lactate]/[Pyruvate]	9.57 ± 0.96	100.94 ± 15.33	52.91 ± 5.50	—	Lactate dehydrogenase (EC 1.1.1.27)
[α-Glycerophosphate]/[Dihydroxyacetone phosphate]	3.55 ± 0.40	76.44 ± 5.75	28.20 ± 2.93	—	α-Glycerophosphate dehydrogenase (EC 1.1.1.8)
[Glucose-6-phosphate] [ADP]/[Glucose] [ATP]	0.01 (2)	0.03 (3)	0.06 ± 0.007	3 900	H[illegible]kinase (EC [illegible] 1.1)
[Fructose-6-phosphate]/[Glucose-6-phosphate]	0.25 ± 0.03	0.26 ± 0.01	0.29 ± 0.02	0.43( )	Phosph[illegible] isomerase (EC [illegible] 1.9)
[Fructose-1,6-diphosphate] [ADP]/[Fructose-6-phosphate] [ATP]	0.10 ± 0.01	0.45 ± 0.04	0.45 ± 0.10	910( )	Phosphof[illegible]kinase (EC 2.7.1.11)
[Glyceraldehyde-3-phosphate]/[Fructose-1,6-diphosphate]/[Fructose 1,6-diphosphate]	0.09 ± 0.03 × 10^{-4}M	0.43 ± 0.08 × 10^{-4}M	0.18 ± 0.07 × 10^{-4}M	0.99 × 10^{-4}M( )	Fructose diphosphate aldolase (EC 4.1.2.13)
[Dihydroxyacetone phosphate]/[Glyceraldehyde 3-phosphate]	8.90 ± 0.44	12.70 ± 1.40	14.33 ± 1.83	22(2)	Triose phosphate isomerase (EC 5.3.1.1)
[2-Phosphoglycerate]/[3-Phosphoglycerate]	0.20 ± 0.06	0.30 ± 0.07	0.22 ± 0.04	0.17(1)	Phosphoglycerate mutase (EC 5.4.2.1)
[Phosphoenolpyruvate]/[2-Phosphoglycerate]	3.54 ± 0.21	1.61 ± 0.24	0.92 ± 0.07	2.8(1)	Enolase (EC 4.2.1.11)
[Pyruvate] [ATP]/[Phosphoenolpyruvate] [ADP]	1.76 ± 0.46	1.09 ± 0.22	2.60 ± 1.06	20,000(1)	Pyruvate kinase (EC 2.7.1.40)
[ATP] [AMP]/[ADP]^2	0.97 ± 0.17	1.03 ± 0.24	0.55 ± 0.09	0.94—1.03(3) depends on [Mg^{++}]	Adenylate kinase (EC 2.7.4.3)

phoglucose isomerase, phosphoglycerate mutase, enolase and adenylate kinase were close to equilibrium while those catalysed by hexokinase phosphofructokinase and pyruvate kinase were far from equilibrium, both *in vivo* and under anaerobic conditions. The triose phosphate isomerase and fructose diphosphate aldolase reactions were not in equilibrium *in vivo* but under anaerobic conditions moved towards equilibrium. The initial rate of lactate production in these experiments was 2.56 ± 0.24 μmol/min/g wet wt., which is very similar to that reported from this laboratory (47)

Intermediates of glycolysis in livers perfused with D-fructose

Livers from well fed rats were perfused in the presence of 1 mM HCN with a medium containing D-fructose (initial concentration, 10 mM) The livers were freeze-clamped after 40 min of perfusion and the perfusion medium was sampled at intervals to determine the time course of changes in the concentration of lactate, pyruvate glucose and D-fructose.

The contents of intermediary metabolites in fructose-perfused livers are shown in Table VIII and comparison with values obtained for livers freeze-clamped *in vivo* shows the following

Adenine nucleotides and inorganic phosphate and IMP The total adenine nucleotide content decreased to 53 % of the *in vivo* value, this was mainly due to the fall in ATP content to 23 % of the *in vivo* value, the ADP content decreased a little to 90 % of the *in vivo* value and the AMP content rose about two-fold The P_i content did not change significantly The fall in the total adenine nucleotide content was accompanied by a three-fold rise in the content of IMP to 0.52 μmol/g wet wt. This is higher than the IMP content during anaerobic perfusion in the absence of added substrate and suggests that deamination of AMP to give IMP was more rapid in the presence of D-fructose

Lactate and pyruvate The content of lactate rose seven fold to 5.62 μmol/g wet wt., which is higher than the content found in livers from well fed rats perfused under identical conditions in the absence of D-fructose. The pyruvate content rose 13 % to 0.11 μmol/g wet wt. and the [lactate]/[pyruvate] ratio rose to 52.9 which is half the value found in anaerobic livers perfused in the absence of D-fructose.

Other phosphorylated intermediates. The content of the phosphorylated intermediates were much the same as those found in livers perfused in the absence of D-fructose in that the intermediates above the triose phosphate level of the glycolytic sequence rose while those below fell. The one exception to this general statement was the content of 3-phosphoglycerate, which rose 12 % to 0.05 μmol/g wet wt. in livers perfused with D-fructose. The triose phosphates both rose dihydroxyacetone phosphate four fold and glyceraldehyde-3-phosphate two-fold.

Fructose-1 phosphate rose twentyfour fold to 5.54 μmol/g wet wt. and α-glycerophosphate to 4.40 μmol/g wet wt.

Other intermediates The glucose content did not change significantly and the free fructose content at the time of freeze-clamping was 2.68 μmol/g wet wt. The D-glyceraldehyde content was 0.71 μmol/g wet wt. and the citrate content fell to 0.19 μmol/ wet wt.

The balance and rates of anaerobic D fructose metabolism. The sum of the products found during the anaerobic perfusion of livers from well fed rats is shown in Table X. The balance refers to the perfusion medium and liver in experiments where the livers were

Table X. *Balance of D-fructose metabolism in the anaerobic perfused rat liver*

The amounts of products found represent the sum of metabolites in medium and liver found after 40 min of perfusion in the presence of 1mM HCN and an initial concentration of 10 mM D-fructose. The quantities of most other intermediates in medium and liver (listed in T ble VIII) were small and have been omitted from the calculation f the balance. Results are all expressed as μmol.

Liver wt.	Fructose removed	F 1 P	α-GP	Lactate	Pyruvate	Glucose	Sum of products (expressed in C_3 units)
		Products found					
7.35	845	42	36	1351	30	534	1304
8 42	575	65	46	1043	15	425	1042
8.12	528	31	31	869	7	762	1248
9 45	751	38	41	1525	7	1270	2095
8.95	582	64	26	1036	18	538	1131

freeze-clamped after 40 min. If the assumption is made that all the fructose passes through the F 1 P stage, some 7.3 / of the fructose metabolised accumulated as F 1 P in the liver The initial rates of metabolic change obtained for the experiments (Table XI) are of the same order of magnitude as those given by Woods and Krebs (47) for livers from well-fed rats perfused under identical conditions.

Metabolite ratios The metabolite ratios for some of the reactions of glycolysis (Table IX) were similar to those measured in livers freeze-clamped in the absence of fructose The reactions catalyzed by hexokinase, phosphofructokinase and pyruvate kinase were far from equilibrium the triose phosphase isomerase and fructose diphosphate aldolase reaction ratios moved closer to equilibrium under anaerobic conditions in the presence of D-fructose.

The distribution of metabolites between liver and perfusion medium

Comparison of the metabolite concentrations in the medium with those in the liver after correction for the metabolite content and volume f the entrapped medium, as described above, shows that gradients exist for glucose, fructose and lactate (Table XII). In both groups of experiments the hepatic glu cose content was higher than that in the medium, the difference being 3 48 mM in the absence of added substrate and 2.46 mM in the presence of fructose. The concentration of fructose in the medium was 3.04 mM greater than that in the liver The lactate content of the liver the site of production, was lower than that in the medium. In the absence of added substrate this gradient was small (0 88 mM) and was not statistically significant

Table XI. *The rates of metabolic changes during anaerobic perfusion*

The initial rates f metabolic changes during anaerobi perfusion of livers from well fed rats perfused with D-fructose (initial concentration 10 mM) were calculated from plot of total metabolites in the perfusion medium versus time. The results are means ± S.E.M. for five observations. The experiments were the same as those in Table.

Metabolite change	Initial rate (μmol/min/g wet wt.)
Fructose removed	2.24 ± 0.24
Glucose formed	2.07 ± 0.37
Lactate formed	3 29 ± 0.33
Pyruvate formed	0.05 ± 0.008

Table XII. *Metabolite concentrations in freeze-clamped liver and perfusion medium*

The values for the metabolite content of liver have been corrected for the metabolite content and volume of the extracellular space as described in the text. The data refers to livers from well-fed rats freeze-clamped after 40 min of anaerobic perfusion in the presence of 1mM HCN The results are mean ± S.E.M. with the number of observations in parentheses.

		Metabolite concentration (mM)		Metabolite ratio in medium / Metabolite in tissue (corrected)
Condition	Metabolite	Perfusion medium	Freeze-clamped liver (corrected)	
Livers perfused in the absence of added substrate	Glucose	6.59 (2)	10.07 (2)	0 65
	Lactate	5.28 ± 0 53 (5)	4 40 ± 0.67 (5)	1.20
	Pyruvate	< 0.01 (5)	0 05 ± 0.008 (5)	—
Livers perfused with D-fructose (initial concentration 10 mM)	Fructose	5 00 ± 0 40 (5)	1.96 ± 0.20 (5)	2.55
	Glucose	5.26 ± 1 05 (5)	7 72 ± 2.00 (5)	0 68
	Lactate	7 61 ± 0.70 (5)	5.02 ± 0 39 (5)	1.52
	Pyruvate	0.10 ± 0 03 (5)	0.11 ± 0 01 (5)	0.91

($p > 0.2$) but it rose to 2.59 mM in the presence of fructose, a difference in concentration which was statistically significant ($p < 0\ 01$)

DISCUSSION

Adenine nucleotide depletion after fructose loading

The experiments confirm that when the rat liver is loaded with fructose, either by intravenous injection of fructose or the addition of fructose to the medium perfusing the isolated organ, there is a rapid loss of hepatic adenine nucleotides (27 32) accompanied by a large accumulation of fructose-1 phosphate At 10 min after fructose loading some 36 % of the original adenine nucleotides were recovered as IMP

The hepatic changes after fructose loading can be explained on the basis of the properties of the enzymes of fructose metabolism and of adenine nucleotide degradation The primary step as suggested by Maenpaa et al. (27) is the rapid phosphorylation of fructose to fructose-1 phosphate catalyzed by ketohexokinase. This decreases the concentration of ATP and P_i, both of which are essential in stabilizating AMP and therefore the total adenine nucleotide content of the liver They inhibit the enzymes that cause the virtually irreversible degradation of AMP P_i inhibits AMP deaminase (30) and at high concentrations 5-nucleotidase (40) which is also inhibited by ATP (2) When the inhibition of AMP deaminase becomes less effective the hepatic IMP concentration rises and AMP and IMP are dephosphorylated with the eventual formation of uric acid and allantoin

In addition to fructose, loading the liver with glycerol (8 48) or xylitol (48) also results in adenine nucleotide depletion and the accumulation of large amounts of α-glycerophosphate. The activation of hepatic AMP degrading enzymes as outlined above might be expected to occur during the hepatic metabolism of any substrate involving a rapid phosphorylation step and the accumulation of a phosphorylated intermediate. This would result in a lowering of the hepatic P content

Table XIII. *Properties of enzymes of fructose metabolism in rat liver*

The results refer to normal well-fed rats but the strains and diets used by different authors were not identical. The assays of ketohexokinase in this laboratory were carried out as described by Adelman et al. (1) and those of ketose-1 phosphate aldolase as described previously (46)

	Ketohexokinase	Ketose-1 phosphate aldolase	References
Maximal rates (μmol/min per g of tissue) at 25°C and pH 7.4—7.5, mean ± S.D. and number of observations	2.12		(1)
	3.14 ± 0.81 (7)	3.40 ± 0.36 (3)	(46)
	2.20 ± 0.39 (6)	1.63 ± 0.11 (5)	(16)
K_m (mM) (mean, S.D. and no. of observations)	0.2 — 0.5		(1)
	0.4		(31)
		0.35 ± 0.18 (6)	(46)

because the sequestration of phosphate as the intermediate is greater than the amount of P liberated by the dephosphorylation of the adenine nucleotides. This hypothesis is confirmed by the finding that loading with dihydroxyacetone, which does not lead to adenine nucleotide depletion, does not result in hepatic accumulation of dihydroxyacetone phosphate or other phosphorylated intermediate (9, 48).

The accumulation of fructose-1 phosphate

The accumulation of fructose-1 phosphate after fructose loading has been known since 1942 (22) the reasons for the accumulation have hitherto not been understood. It could not be explained on the basis of the relative activities of ketohexokinase and ketose-1 phosphate aldolase because under the conditions in the liver *in vivo* the activities of the two enzymes are about the same (Table XIII). After fructose loading, when the concentration of free fructose is lower than that of fructose-1 phosphate, the activity of ketohexokinase may be expected to be lower than those of ketose-1 phosphate aldolase. The finding that the IMP which accumulates strongly inhibits ketose-1 phosphate aldolase fully accounts for the accumulation of fructose-1-phosphate.

The anaerobic metabolism of fructose

The rapid metabolism of fructose and the hepatic accumulation of fructose-1-phosphate during the anaerobic perfusion (Table VIII) indicate that the conditions are favourable for hepatic fructokinase activity. Dihydroxyacetone phosphate and D-glyceraldehyde, the products of the fructose-1-phosphate aldolase reaction, did not accumulate to any great extent and thus the reactions subsequent to this step must have been rapid. The phosphorylation of D-glyceraldehyde to glyceraldehyde-3-phosphate by the triokinase (EC 2.7.1.28) reaction is favoured because of the low K_m for D-glyceraldehyde in this reaction (0.01 mM, ref. 41), and some 90 % of the D-glyceraldehyde formed from fructose in the liver is metabolised in this way (41). An alternative pathway of D-glyceraldehyde metabolism is conversion to 2 phosphoglycerate by oxidation to glycerate by aldehyde dehydrogenase (EC 1.2.1.3) and subsequent metabolism via glycerate kinase (EC 2.7.1.3). In the absence of added substrate the hepatic 2-phosphoglycerate content fell. In contrast it rose in the fructose-perfused anaerobic livers. This may have been due to the metabolism of some D-glyceraldehyde via the glycerate pathway.

Pyruvate kinase is activated by fructose-1-phosphate at concentrations which occur in rat liver during perfusion with 10 mM fructose (14) Anaerobic lactate formation from fructose (via pyruvate kinase) is more rapid than that from endogenous glycogen (47) The maximum rate of lactate formation from fructose in the perfused liver is up to 4 μmol/min/g wet wt. (47) and the activity of pyruvate kinase when assayed at physiological concentrations of phosphoenol pyruvate is of the same order (14) It is therefore to be postulated that the activation of pyruvate kinase by fructose-1 phosphate is essential for maximal rates of anaerobic fructolysis.

ACKNOWLEDGEMENTS

I gratefully acknowledge the helpful criticism of this manuscript by Professor H. A. Krebs, and the collaboration of Professor Krebs and Mr L. V Eggleston in the work.

Tables I II, III, IV V VI, XIII are reproduced with the permission of the Editorial Board of the Biochemical Journal.

REFERENCES

1 Adelman, R. C., Ballard, F J & Weinhouse, S Purification and properties of rat liver fructokinase. J biol. Chem. 242 3360 1967

2 Baer H. P Drummond, G I. & Duncan, E. L. Formation and deamination of adenosine by cardiac muscle enzymes. Mol. Pharmacol. 2 67 1966.

3. Baranowski, T Crystalline glycerophosphate dehydrogenase from rabbit muscle. J biol. Chem. 180 535 1949

4. Brdiczka, D Pette D Brunner G & Miller F Kompartimentierte Verteilung von Enzymen in Rattenlebermitochondrien. Eur J Biochem. 5 294, 1968

5. Brosnan, J T Krebs, H A & Williamson, D H. Effects of ischaemia on metabolite concentrations in rat liver Biochem J 117 71 1970

6. Bucher R., Krejci, K Russman, W Schnitger H. & Weseman, W I Rapid Mixing and Sampling Techniques in Biochemistry p. 255. Ed. by Chance, B Eisenhardt, R. E., Gibson, Q H. & Lonberg Holm, K. K. Academic Press Inc., New York, 1964

7 Bunag, R. D Douglas, C. R., Imae S & Berne, R. M. In vitro inhibition of adenosine deminase by persantin. Fed. Proc. 22 2876, 1963.

8. Burch, H. B., Lowry O H., Meinhardt, L., Max, P & Chyu, K. Effects of fructose, dihydroxyacetone, glycerol and glucose on metabolites and related compounds in liver and kidney J biol. Chem. 245 2092, 1970.

9 Burch, H. B., Max, P Chyu, K. & Lowry O H. Metabolic intermediates in liver of rats given large amounts of fructose or dihydroxyacetone. Biochem. biophys. Res. Commun. 34 619 1969

10 Burton, K Free energy data of biological interest. Ergebn. Physiol. 49 275, 1957

11 Chappell, J B & Crofts, A. R. The effect of atractylate and oligomycin on the behaviour of mitochondria towards adenine nucleotides Biochem. J 95 707 1965.

12. Dickens, F & Greville, G D The metabolism of normal and tumour tissue. The anaerobic conversion of fructose into lactic acid by tumour and adult normal tissue. Biochem. J 26 1546, 1932.

13. Dixon, M. The determination of enzyme inhibitor constants. Biochem. J 55 170, 1953

14 Eggleston, L. V & Woods, H. F Activation of liver pyruvate kinase by fructose 1-phosphate. FEBS Letters 6 43, 1970.

15 Heinz, F Enzymatische Bestimmung von Fructose 1 phosphat. Hoppe-Seyler's Z. physiol. Chem. 349 859 1968.

16 Heinz, F Lamprecht, W & Kirsch, J Enzymes of fructose metabolism in human liver J clin. Invest. 47 1826, 1968.

17 Hems, D A. & Brosnan, J T Effects of ischaemia on content of metabolites in rat liver and kidney in vivo. Biochem. J 120 105 1970

18. Hems, R., Ross, B. D Berry M. N & Krebs, H. A. Gluconeogenesis in the perfused rat liver Biochem. J 101 284 1966.

19 Hers, H. G La fructokinase du foie. Biochim. biophys Acta 8 416, 1952.

20 Hurlbert, R. B Schmitz, H Brumm, A. F & Potter V R. Nucleotide metabolism. II Chromatographic separation of acid-soluble nucleotides. J biol. Chem. 209 23, 1954

21 Judah, J D Ahmed, K., M Lean, E. A. K & Christie G S Ion transport in ethionine intoxication Lab Invest. 15 167 1966.

22. Kjerulf-Jensen, K. The phosphate esters formed in the liver tissue of rats and rabbits during assimilation of hexoses and glycerol. Acta physiol. scand. 4 249 1942.

23. Krebs, H. A., Freedland, R. A., Hems, R. & Stubbs, M. Inhibition of hepatic gluconeogenesis by ethanol. Biochem. J 112 117 1969

24. Krebs, H. A. & Henseleit, K. Untersuchungen über die Harnstoffbildung im Tierkörper Hoppe-Seyler's Z. physiol. Chem. 210 33, 1932.

25. Leuthardt, F & Testa, E. Die Phosphorylierung des Fructose in der Leber Helv chim Acta 34 931, 1951.

26. Lineweaver H. & Burk, D The determination of enzyme dissociation constants. J Am Chem. Soc. 56 658, 1934

27 Mäenpää, P H., Raivio, K. O. & Kekomaki, M. P., Liver adenine nucleotides fructose induced depletion and its effect on protein synthesis. Science N Y 161 1253, 1968.

28. Miller M., Drucker W R., Owens, J E Craig, J W & Woodward, H. J Metabolism f intravenous fructose and glucose in normal and diabetic subjects J clin Invest 31 115 1952.

29. Murray A. W Purine phosphoribosyltransferase activities in rat and mouse tissues and in Ehrlich ascites-tumour cells Biochem. J 100 664, 1966.

30 Nikiforuk, G. & Colowick, S. P The purification and properties of 5-adenylic acid deaminase from muscle. J biol. Chem 219 119 1956

31 Parks, R. E., Ben-Gershom, E. & Lardy H A Liver fructokinase. J biol. Chem. 227 231 1957

32 Raivio, K. O., Kekomaki, M P & Maenpaa, P H. Depletion f liver adenine nucleotides induced by D-fructose, dose-dependence and specificity of the fructose effect. Biochem. Pharmac. 18 2615, 1969

33. Rose, I. A. The state of magnesium in cells as estimated from the adenylate kinase equilibrium. Proc N t. Acad Sci U S A. 61 1079 1968.

34. Rosenthal, O Untersuchungen über Milchsäuregärung von Warmblütergeweben die Vergärbarkeit verschiedener Zuckerarten durch Rattenleber Ihr Abhängigkeit vom Ernährungszustand des Versuchstiers und von jahreszeitlichen Einflüssen Biochem. Z. 227 354, 1930 (a).

35 Rosenthal, O Untersuchungen zum Spaltungsstoffwechsel von geschwülsten und normalen Geweben. Z. Krebsforsch. 32, 220, 1930 (b).

36. Rosenthal, O Die Beziehungen zwischen spontaner Extragärung und Zuckervergärung bei der Leber Biochem. Z. 233 62, 1931

37 Rutter W J Woodfin, B. M. & Blostein, R. E. Enzymi homology Structural and catalyti differentiation of fructose diphosphate aldolase. Acta chem. scand. 17 Suppl. 1 226, 1963.

38. Schmitz, H., Hurlbert, R. B. & Potter V R., Nucleotide metabolism. III. Mono- di and triphosphates of cytidine guanosine and uridine J biol. Chem. 209 41, 1954.

39 Schwimmer S. Kinetics of malt α-amylase action. J bi l. Chem. 186 181 1950

40 Segal, H. L. & Brenner B M. 5-nucleotidase of rat liver microsomes. J biol. Chem. 235 4 1, 1960.

41 Sillero, M. A. G Sillero, A. & Sols, A., Enzymes involved in fructose metabolism in liver and the glyceraldehyde metabolic crossroads. Eur J Biochem. 10 345, 1969

42 Sols, A. & Crane R. K. Substrate specificity of brain hexokinase. J biol. Chem. 210 581, 1954

43. Spolter P D Adelman, R. C. & Weinhouse, S. Distinctive properties of native and carboxypeptidase-treated aldolases of rabbit muscle and liver J biol. Chem. 240 1327 1965

44 Weech, R. L., Raijman, L., Dalziel, K. & Krebs, H. A. Disequilibrium in the triosephosphate isomerase system in rat liver Biochem. J 115 837 1969

45. Walker D G On the presence of two solubl glucose-phosphorylating enzymes in adult liver and the development of one of these after birth. Biochim. biophys. Acta 77 209 1963.

46. Woods, H. F Eggleston, L. V & Krebs, H. A. The cause f hepati accumulation of fructose 1-phosphate on fructose loading. Biochem. J 119 501, 1970.

47 Woods, H. F & Krebs, H. A. Lactate production in the perfused rat liver Biochem. J 125 129 1971.

48. Woods, H. F & Krebs, H. A. In preparation.

Acta med. scand. Suppl. 54.., pp. 105—110

EFFECT OF FRUCTOSE ON CELLULAR RESPIRATION IN PERFUSED RAT LIVER

L. E. Hassinen, R. H. Ylikahri and M. T. Kähönen

From the Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Abstract. 1. The regulation of energy metabolism is reviewed with special reference to the metabolism of fructose. Fructose metabolism in liver by-passes the main regulatory step of glycolysis, the formation of fructose-6-phosphate. Therefore the overall regulation of energy metabolism is possibly adjusted in a new way. The by-pass and the high activity of liver fructokinase cause metabolic alterations characterized by an accumulation of fructose-1-phosphate and depletion of inorganic phosphate and adenine nucleotides in the liver.

2. The effects of fructose on the redox state of nicotinamide nucleotides and mitochondrial flavoproteins and cytochromes were studied by surface fluorometry and transmission spectrophotometry in isolated perfused rat liver.

Oxygen consumption was inhibited and nicotinamide nucleotides were reduced concomitant with phosphate depletion. The spectrophotometry of intact tissue showed inhibition of electron transport at the first phosphorylation site of the mitochondrial respiratory chain.

The fructose-induced alterations in the cellular redox state can be interpreted as acceptor control for inorganic phosphate during the metabolism of fructose. The role of the metabolism of glyceraldehyde in these redox changes is discussed.

The metabolism of fructose in the liver has some unusual characteristics: 1) it takes place at a high rate compared with glucose metabolism (22), 2) it involves several metabolic crossroads not shared by glucose metabolism (4, 13, 15), 3) this suggests that the control mechanisms of fructose metabolism may be different from those of glucose metabolism. Fructose is mainly phosphorylated to fructose-1-phosphate, which is directly attacked by aldolase B (fructose-1-phosphate aldolase) to yield D-glyceraldehyde and dihydroxyacetone phosphate (4, 20). Thus, the rate of fructose catabolism in the liver is not influenced by phosphofructokinase which is considered to be the most important regulatory enzyme in glycolysis (27). It might be expected that this lack of anaerobic control of fructose breakdown would be reflected in a typical pattern of effects on aerobic energy metabolism. We shall refer to the latter as cellular respiration. This includes the terminal oxidations in the tricarboxylic

acid cycle and respiratory chain with the energy conservation system.

The aerobic fate of the trioses formed from fructose in the liver is probably similar to the fate of the three-carbon compounds formed in glycolysis. The formation of glycerophosphate (2) from fructose is not reflected in the respiratory rate because the activi ty of the mitochondrial glycerophosphate dehydrogenase is very low (14).

Since fructose evades the major regulatory step of glycolysis, the mutual regulation of glycolysis and respiration would be expected to become seriously unbalanced. This normal integration of glycolysis and respiration is shown by the Pasteur and Crabtree effects (5 18) The main mechanisms in the control of respiration are the acceptor control of the mitochondrial respiratory chain for ADP and inorganic phosphate (3) the adenylate con trol of the tricarboxylic acid cycle (1) and the control of the tricarboxylic acid cycle by the redox state of mitochondrial nicotinamide adenine nucleotides (28) Obviously the over all control of energy metabolism involves a omplex network of these mechanisms.

This communication reviews briefly the fructose-induced changes in cellular respira tion. It also describes some effects of fruc tose on the intracellular redox state and on regulation by the mitochondrial electron transport carriers These were studied by sensitive fluorometric and spectrophotometric methods in intact tissues.

EFFECT OF FRUCTOSE ON THE OXYGEN CONSUMPTION OF THE LIVER

When 5 to 10 mM fructose is added to the perfusate of a liver perfusion, there is a rapid initial stimulation of oxygen consumption lasting 2 to 3 minutes This stimula tion is followed by an inhibition reaching a maximum after 6 to 7 minutes In liver from a fed rat respiration drops to 70 of the steady state value (9) but in starved rat liver

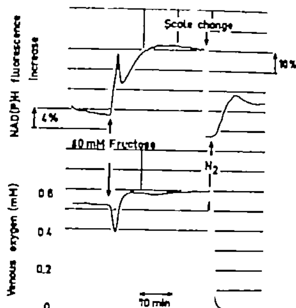


Fig. 1 Effect of fructose on the redox state of liver nicotinamide nucleotides. NAD(P)H fluorescence (upper curve) was recorded by surface measurement of an isol ted perfused rat liver Perfusion medium Krebs—Ringer bicarbonat buffer pH 7 4, 35 flow rate 30 ml/min. Fructose (10 mM) was added to the perfusion medium at the injection port before the xygenator to avoid oxygen tension artifacts. Oxygen concentration in the outlet cannula in the inferior caval vein was monitored with Clark type oxygen electrode Arterial oxygen concentration was kept constant t 1 01 mM, except under the cycle of anoxia induced by changing the gas phase (95 /, O_2, 5 / CO_2) to 95 / N_2, 5 % CO_2 t the time indicated by N_2 The rat fasted 20 hrs before the experiment. The fluorometer used fibre optics for both the excitation and emission light (7). Excitation light wavelength (365 nm) was isolated from a low-pressure mer cury rc lamp and modulated at 50 Hz. The multiplier phototube was guarded against excitation light by a Kodak Wratten 2A filter Zero suppression was with the excitation light intensity as a reference signal. Percentage numbers refer to the initial fluorescence of the tissue.

the inhibition is smaller (Fig 1) Occasionally inhibition persists, but usually it fades after 10 minutes The pattern of the respiratory inhibition is also affected by the thyroid state of the animal (11)

EFFECT OF FRUCTOSE ON THE INTRA CELLULAR REDOX STATE

Both the stimulatory and inhibitory phases are accompanied by reduction of nicotina mide nucleotides. The time course of this effect is easily demonstrated by measuring the NAD(P)H fluorescence of whole tissue (Fig. 1). There is a small reoxidation at the time of the transition from stimulation to inhibition of respiration. This redox change is reflected in the NAD-linked redox substrate pairs, for instance there is a rise in the lactate/pyruvate (L/P) concentration ratio (2, 12, 19). The time course of the changes in L/P ratio has not been studied in detail by rapid sampling. However samples taken by Raivio et al. (19) at 1 2, 5 and 10 min. showed an L/P ratio maximum at 5 min. This is the opposite to the oxidative effect which the sorbitol dehydrogenase and glyceraldehyde reductase (alcohol dehydrogenase) enzymes could be expected to exert. If these two enzymes are functioning in the metabolism of fructose, their effects must be swamped by other effects, such as NADH formation in glycolysis or perhaps effects at the level of mitochondrial oxidations

We tested the latter possibility by studying the redox changes of mitochondrial respiratory carriers under these same conditions (9). The redox state of the fluorescent mitochon drial flavoproteins shifted towards oxidation during the stimulation of respiration and towards reduction during the inhibition of respiration (Fig 2). The fluorescent flavoprotein of intact liver tissue has been identified as the lipoic dehydrogenase (8, 24), *i.e.* the flavoprotein component of the α-oxoacid dehydrogenase complex. This flavoprotein is in equilibrium with the mitochondrial NADH/NAD pool, and thus is an indicator of the redox state of mitochondrial nicotinamide adenine nucleotides in intact tissue (24). Comparison of the traces of Figs. 1 and 2 shows a dissociation between the redox transitions

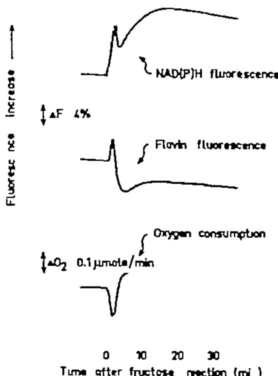


Fig 2 Effect of fructose on the respiration and redox state of the nicotinamide nucleotides and flavoproteins in isolated perfused rat liver Experimental conditions as in Fig. 1. Fructose (10 mM) was added to the perfusion medium t time 0 Flavoprotein fluorescence was measured by using the 436 nm mercury line for fluorescence excitation. The multiplier phototube was guarded against excitation light by a broadband filter peaking at 532 nm with half intensity band width f 57 nm. Oxygen concentration in the venous cannula was monitored with an oxygen electrode. The two upper curves are means of three experiments, the lowest of six experiments. Shaded area represents mean ± SEM.

of cytosol (as reflected in NADH fluorescence) and mitochondria (as reflected in flavin fluorescence) This is somewhat puzzling because at least during the ethanol induced redox change of NADH/NAD the changes in cytosol and mitochondria are roughly parallel (11 24). However this dis-

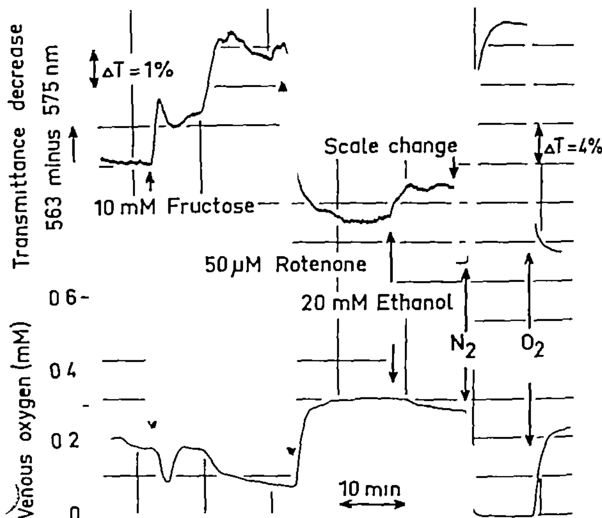


Fig 3 Effect of fructose on the redox state of cytochrome b and respiration in an isolated perfused rat liver Experimental conditions as in Fig 1 Fructose (10 mM) was added at the time indicated Thereafter the cytochrome was completely oxidized by adding rotenone (50 µM) and completely reduced by anoxia (as in Fig 1) The upper curve represents the difference of transmittances at 563 and 575 nm recorded by a dual wavelenght spectrophotometer An upward deflection means reduction of cytochrome b Oxygen concentration (lower curve) in the venous cannula was monitored with an oxygen electrode.

sociation is only evident during the rapid initial changes. The long term effects in both compartments are towards reduction.

EFFECTS OF FRUCTOSE ON THE MITOCHONDRIAL RESPIRATORY CHAIN

The behaviour of the mitochondrial redox state suggests a control on the oxygen side of the mitochondrial NADH/NAD pool. This is highly probable since the spectrophotometrically measurable redox changes in the mitochondrial cytochrome chain showed a control point at this level (9 10) The most clearcut redox changes were in cytochrome b These changes were almost a mirror image of the changes in the redox state of the fluorescent flavoproteins (Fig 3). The redox transitions of cytochrome cc_1 and cytochrome aa_3 were much more difficult to interpret (9)

GENERAL DISCUSSION OF THE CONTROL OF FRUCTOSE METABOLISM

The results described so far can be explained by inhibition of respiration at the level of the mitochondrial respiratory chain during fructose metabolism.

The respiratory inhibition is most likely to be caused by the changes in the intracellular adenylate compounds and inorganic phosphate brought about by fructose (2, 17 19) This finding was originally made by Maenpää et al. (17) and will be discussed elsewhere in this Symposium. The experiments have been repeated with perfused rat livers The results were similar in principle the only difference being the smaller change in adenine nucleotides in spite of a drastic change in inorganic phosphate (6, 18) The changes in inorganic phosphate kept in pace with the transitions of the respiratory rate (11). It is likely that the depletion of phosphate is caused by a rapid glycolytic and oxidative phosphorylation of the ADP formed in the phosphorylation of fructose to fructose-1-phosphate (2). One tempting explanation is that the respiratory inhib tion is an expression of a mutual control of glycolysis and respiration. In other words, this is a phenomenon very similar to the Crabtree effect observed in ascites tumour cells (5). In fact, phosphate control of glycolysis and respiration has been demonstrated in ascites tumour cells (16, 29)

The physiological significance of the effects described remains to be established The results may only be valid for parenteral administration of fructose. The absorption of fructose from the intestine is quite slow judging by the blood fructose levels found after large oral doses (30) Intravenous fructose decreases serum phosphate levels in man (26). This might indicate that the phosphate control is not specific to one species.

The redox state of nicotinamide adenine nucleotides after fructose administration behaves in accordance with the results of Sillero et al (25) They found that the enzyme profile of the liver and the kinetic parameters of the enzymes involved in the metabolism of glyceraldehyde indicate that the phosphorylation of glyceraldehyde by thiokinase is the main pathway The isotope distribution data obtained with isotopically labelled fructose also argue against the significance of the reductive pathways (reduction by alcohol dehydrogenase to glycerol) (21)

The phosphate control of respiration after fructose loading seems to be one of the few situations in which mitochondrial respiratory control can be demonstrated in intact tissue. For this reason it may develop into a tool for investigating the degree of coupling between oxygen consumption and energy conservation in intact mammalian tissue.

REFERENCES

1 Atkinson, D C. Citrate and the citrate cycl in the regulation of energy metabolism, in Th Metabolic Role of Citrate, Goodwin, T W (ed.), Academic Press, London, 1968, p 23.

2 Burch, H. B Lowry D. H Meinhard, L., Max, P J & Chuy K. Effects of fructose, dihydroxyacetone, glycerol and gl cose on metabolites and rel ted compounds in liver and kidney J biol. Chem. 245 2092, 1970.

3 Chance, B & Williams, G R. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J biol. Chem. 217 383, 1955.

4. Cori, G T Ochoa, S., Slein, M. W & Cori, C. F The metabolism of fructose in the liver Isolation of fructose-1 phosphate and inorganic pyrophosphate. Biochim. biophys. Acta 7 304, 1951

5 Crabtree N G Observations on the carbohydrate metabolism of tumours. Biochem. J 23 536, 1929

6 Exton, J H. & Park, C. R. Control of gluconeogenesis in liver III. Effects of L-lactate pyruvate fructose, glucagon, epinephrine and adenosine 3' 5'-monophosphate on gluconeogenic intermediates in the perfused rat liver J biol. Chem. 244 1424, 1969

7 Hassinen, I. T be published elsewhere.

8. Hassinen, I. & Chance, B. Oxidation-reduction properties of the mitochondrial flavoprotein chain. Biochem. biophys. Res. Commun. 31 895, 1968.

9 Hassinen, I. & Ylikahri, R. H.. Absorption spectrophotometry of perfused rat liver applied to fructose-induced inhibition of respiration. Biochem. biophys. Res. Commun 38 1091 1970

10. Hassinen, I. E., Ylikahri, R. H. & Kähonen, M. T.. Regulation of cellular respiration by thyroid hormone. Spectroscopic evidence of mitochondrial control in intact rat liver Arch. Biochem. Biophys. 147 255 1971

11. Hassinen, I. E., Ylikahri, R. H. & Kähönen, M. T Effect f ethanol, thyroxine and fructose on the intracellular redox state of a perfused liver as studied by surface fluorometry Ann. Med. exp. Fenn. 48 176 1970

12. Heinz, F & Junghänel, J Metabolitmuster in Rattenleber nach Fructoseapplikation. Hoppe-Seyler's Z. physiol. Chem. 350 859 1969

13 Holzer H. & Schneider S. Zum Mechanismus der Beeinflussung der Alkoholoxydation in der Leber durch Fructose. Klin. Wschr 33 1006, 1955

14 Kadenbach, B. Der Einfluss von Thyroid hormonen in vivo auf di oxidative Phosphorylierung und Enzymaktivitäten in Mitochondrien. Biochem. Z. 344 49 1966.

15. Leuthardt, F Testa, E. & Wolf, H P Der enzymatische Abbau des Fructose-1 phosphats in der Leber H lv chim. Acta 36 227 1953.

16 Maitra, P K. & Chance B. ADP and P control in ascites tumor cells, in Control of Energy Metabolism, Chance B., Estabrook, R. W nd Williamson, J R. (Eds) Academic Press, New York 1965 p. 157

17 Maenpää, P H Raivio, K. O & Kekomäki, M P Li er adenine nucleotides Fructose induced depletion and its effect on protein synthesis Science 161 1253 1968

18. Pasteur L. Etudes sur la Biere G uthier Villars Paris 1876

19 Raivio, K. O Kekomäki, M. P & Mäenpää, P H. Depletion of li er adenine nucleotides by D-fructose. Dose-dependence and specificity of the fructose effect Biochem Pharmacol 18 *615, 1969

20. Rajkumar T V Woodfin, B. M. & Rutter, W J Aldolase B from (adult) rabbit liver in Methods in Enzymology vol. 9 Colowick, S. P & Kaplan, N O. (Eds.), Academic Press, New York 1966, p. 491.

21 Rauschenbach, P & Lamprecht, W Einbau von ^{14}C markierter Glucose und Fructose in Leberglykogen. Hoppe-Seyler's Z. physiol Chem. 339 277 1964.

22. Renold, A. E., Hastings, A. B. & Nesbett, F B Studies on carbohydrate metabolism in rat liver slices. III Utilization of glucose and fructose by liver from normal and diabetic animals. J biol. Chem. 209 687 1954.

23 Scholz, R. & Bücher Th. Hemoglobin-free perfusion of rat liver in Control of Energy Metabolism, Chance, B., Estabrook, R. W & Williamson, J R. (Eds.) Academic Press, New York 1965, p. 393.

24 Scholz, R., Thurman, R. G Williamson, J R., Chance B. & Bücher Th. Flavin and pyridine nucleotide oxidation-reduction changes in perfused rat liver I. Anoxia and subcellular localization of fluorescent flavoproteins. J biol. Chem. 244 2317 1969

25 Sillero, M. A. G Sillero, A. & Sols, A. Enzymes involved in fructose metabolism in liver and the glyceraldehyde metabolic crossroads. European. J Biochem. 10 345, 1969.

26 Smith, L. H Ettinger R. H. & Seligson, D. A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. J clin. Invest. 32 273, 1953.

27 Underwood, A. H. & Newsholme E. A. Properties of phosphofructokinase from rat li er and their relation to the control of glycolysis and gluconeogenesis. Biochem. J. 95 868, 1965.

28. Wieland, O Matschinsky F Löffler G & Müller V Die Abhängigkeit der Ketonkörperbildung der Leber von DPNH-Spiegel. Biochim. biophys. Acta 53 412, 1961

29 Wu, R. & Racker E. Regulation mechanisms in carbohydrate metabolism. IV Pasteur ffect and Crabtree effect in ascites tumor cells, J biol Chem. 234 1036, 1959

30 Ylikahri, R. H., Kähönen, M. T & Hassinen, I. E. Modification of metabolic effects of ethanol by fructose Acta med. scand., this supplement, pp 141—150

Acta med. scand. Suppl. 542, pp. 111—114

FRUCTOSE AND PURINE METABOLISM

K. O. Raivio, M. Kekomaki and P. Mäenpää

From the Children's Hospital and Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Abstract. The interactions between fructose and purine metabolism are reviewed. Hyperuricemia and increased production of uric acid after fructose administration have been documented in man as well as in the rat. These effects appear to be dependent on the rapid phosphorylation of fructose, mainly in the liver. This results in depletion of intracellular ATP and inorganic phosphate. As a consequence, the adenylate deaminase reaction is deinhibited and increased breakdown of adenine nucleotides to uric acid ensues.

Some considerations of the hazards involved in the clinical use of fructose are presented, and other possible points of interaction between fructose and purine metabolism are discussed.

Some kind of interaction between fructose and purine metabolism was suggested by the observation that in patients with hereditary fructose intolerance and in normal individuals, a rapid intravenous infusion of fructose resulted in hyperuricemia (18). In subsequent studies, the biochemical basis as well as the clinical implications of this phenomenon have been elucidated, and an alteration of purine nucleotide degradation by fructose has been demonstrated. It is not yet clear whether other aspects of purine metabolism, e.g. *de novo* synthesis, are also affected.

The hyperuricemic response to fructose occurs rapidly in normal individuals, with a peak at 15 min after an intravenous injection of 0.5 g/kg (18). The simultaneous increase in the urinary excretion of urate rules out the possibility of renal retention as the basis for the phenomenon (18), even though blood lactate levels also increase rapidly. In accord are the studies of Fox and Kelley (7), which indicated an unaltered clearance of uric acid during fructose-induced hyperuricemia. The renal tubular secretion of urate is known to be inhibited when blood lactate levels are elevated by infusing sodium lactate (25), but this mechanism is not responsible for the rapid rise in serum urate after fructose administration.

Fructose-induced hyperuricemia thus seems to be due to increased formation of uric acid, either through *de novo* synthesis or through breakdown of preformed purine nucleotides. Evidence for the latter possibility has been obtained by studying rats, in which fructose-induced hyperuricemia and hyperallantoinemia also occur (16).

An intravenous injection of 1 mmole of fructose causes a rapid depletion of the adenine nucleotides of rat liver with a maximal decrease of 50 % 5 min after the injection (16 20) This is mainly due to a decrease of ATP levels, and it is accompanied by a more rapid and marked drop in hepatic inorganic phosphate (P_i) concentrations. Both changes are largely normalized by 30 min after the injection. On the other hand, a marked accumulation of fructose-1 phosphate occurs and persists for a more prolonged period (5 9 15) All these alterations can also be demonstrated in an isolated perfused rat liver preparation, when fructose is added to the perfusion medium (24) They as well as fructose-induced hyperuricemia, can be explained on the basis of the known regulatory behavior of certain enzymes of fructose and nucleotide metabolism.

Fructose is rapidly phosphorylated by ketohexokinase (1 10 24) utilising ATP and resulting also in a decrease in P_i levels. These changes allow an increased rate of the AMP deaminase reaction, which is normally inhibited by P_i (17) and by ATP (21) It is probable that the alternate route of AMP degradation through 5 nucleotidase also increases, because this enzyme is normally inhibited by ATP (2) A marked increase in IMP concentration occurs (24) and is probably responsible for an accentuated sequestration of P as fructose-1-phosphate because IMP has been shown to inhibit fructose-1 phosphate aldolase (24) IMP itself is further broken down to inosine hypoxanthine xanthine and finally uric acid If xanthine oxidase activity is inhibited by allopurinol, fructose-induced degradation of adenine nucleotides is manifested in an increased excretion of the oxipurines hypoxanthine and xanthine (7)

The postulated sequence of events explains the specificity of the effects of fructose on purine metabolism Only fructose and L-sorbose are phosphorylated by ketohexokinase at a rapid rate (1) and therefore other hexoses are without effect (20) Ketohexokinase is present only in the liver kidney and intestinal mucosa (1), explaining the lack of effect of fructose on the ATP levels in rat heart (20)

The importance of increased breakdown of preformed purine nucleotides in the causation of fructose-induced hyperuricemia is supported by rough calculations on the basis of liver perfusion experiments. The quantitative increase in allantoin production agreed closely with the estimated loss of adenine nucleotides from the intact liver (20) However a primary stimulation of *de novo* purine synthesis has not been ruled out as a contributing mechanism. In any case, a secondary increase in *de novo* synthesis is to be expected, because the depletion of adenine nucleotides would tend to release the feedback inhibition exerted by these compounds on the rate-limiting enzyme of purine synthesis, 5-phosphoribosyl 1-pyrophosphate amidotransferase. Preliminary data indicate that the incorporation of ^{14}C-glycine into urinary uric acid is increased as a result of fructose infusion, but it has not been possible to evaluate whether this is a primary or a secondary effect (19).

The clinical implications of fructose-induced hyperuricemia deserve comment, because fructose is commonly used in intravenous fluid therapy and is a common constituent of the diet. As discussed above, the elevation of serum and urinary uric acid seems to be a consequence of the breakdown of adenine nucleotides mainly in the liver In patients undergoing abdominal surgery a 50 decrease in liver ATP and ADP levels has in fact been demonstrated by serial biopsy in the course of a 30 min infusion of fructose but not of glucose (4) Interference with protein synthesis is a known manifestation of such a depletion (8, 16), whereas other possible consequences are largely unexplored. In any case infusion of fructose at rates producing hyperuricemia can be considered potentially harmful. Even at lower rates, elevation of blood lactate levels and resulting

changes in acid-base balance must be taken into account (3, 14).

The injection of a single dose of 0.5 g/kg of fructose was followed by a marked increase in serum and urinary urate in children (18) and an oral dose of 1 g/kg had an unequivocal effect in normal and gouty adults (23). On the other hand, a continuous fructose infusion at the rate of 0.5—0.7 g/kg/hr apparently does not influence serum urate levels in adults (6, 11, 22) but at the rate of 1—1.5 g/kg/hr a hyperuricemic response has been demonstrated (11, 14). It is apparent that a significant amount of calories can be supplied intravenously in the form of fructose without provoking significant hyperuricemia. In post operative patients receiving fructose at the rate of 0.3 g/kg/hr a slight increase in urinary uric acid excretion was noted in comparison to patients receiving glucose (12) but this difference was not impressive in comparison to the catabolic effect of surgical trauma per se. On the basis of animal experiments it appears that the increased breakdown of phosphorylated adenine derivatives by anoxia and by fructose administration may be additive in action (13).

It is also apparent that a marked elevation of blood lactate levels occurs even when fructose is administered in amounts not producing changes in serum uric acid levels (22). Therefore, from the clinical standpoint acid base considerations may outweigh possible interference with purine metabolism in evaluations of the usefulness of fructose.

REFERENCES

1. Adelman, R. C., Ballard, F. J. & Weinhouse, S. Purification and properties of rat liver fructokinase. J. biol. Chem. 242 3360, 1967.
2. Baer, H. P., Drummond, G. I. & Duncan, E. L. Formation and deamination of adenosine by mammalian muscle enzymes. Molec. Pharmacol. 2, 67, 1966.
3. Bergström, J., Hultman, E. & Roch-Norlund, A. E. Lactic acid accumulation in connection with fructose infusion. Acta med. scand. 184 359, 1968.
4. Bode, C., Schumacher, H., Goebell, H., Zelder, O. & Pelzer, H. Fructose-induced depletion of liver adenine nucleotides in man. Horm. Metab. Res. 3 289, 1971.
5. Burch, H. B., Max, P. Jr., Chyu, K. & Lowry, O. H. Metabolic intermediates in liver of rats given large amounts of fructose or dihydroxyacetone. Biochem. biophys. Res. Commun. 34 619, 1969.
6. Curreri, P. W. & Pruitt, B. A. Absence of fructose-induced hyperuricemia in men. Lancet I 839, 1970.
7. Fox, I. H. & Kelley, W. N. Mechanism of fructose-induced hyperuricemia in man. Presented at the Annual Meeting of the American Rheumatism Association, San Diego 1971.
8. Goldblatt, P. J., Witschi, H., Friedman, M. A., Sullivan, R. J. & Shull, K. H. Some structural and functional consequences of hepatic adenosine triphosphate deficiency induced by intraperitoneal fructose administration. Lab. Invest. 23 378, 1970.
9. Heinz, F. Enzymatische Bestimmung von Fructose-1-phosphat. Hoppe-Seyler's Z. physiol. Chem. 349 859, 1968.
10. Heinz, F., Lamprecht, W. & Kirsch, J. Enzymes of fructose metabolism in human liver. J. clin. Invest. 47 1826, 1968.
11. Heuckenkamp, P.-U. & Zollner, N. Fructose-induced hyperuricaemia. Lancet I 808, 1971.
12. Kekomäki, M., Collan, R., Lindfors, O. & Raivio, K. Postoperative alimentation with fructose and glucose. Similar effects on urinary excretion of uric acid. Acta chir. scand. 137, 1971.
13. Kekomäki, M., Louhimo, I., Rahiala, E.-L. & Suutarinen, T. Comparison of fructose and glucose solutions in the treatment of hypovolemic shock in rabbits. Acta chir. scand. 137, 1971.
14. Kekomäki, M., Raivio, K. O. & Mäenpää, P. H. Interference with liver metabolism by D-fructose. Acta anaesth. scand. Suppl. 37 114, 1970.
15. Kjerulf-Jensen, K. The phosphate esters formed in the liver tissue of rats and rabbits during assimilation of hexoses and glycerol. Acta physiol. scand. 4 249, 1942.
16. Mäenpää, P. H., Raivio, K. O. & Kekomäki, M. P. Liver adenine nucleotides: Fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.

17 Nikiforuk, G & Colowick, S. P The purification and properties of 5-adenylic acid deaminase from muscle. J biol. Chem. 219 119 1956.

18. Perheentupa, J & Raivio, K. Fructose-induced hyperuricaemia. Lancet II 528, 1967

19 Raivio K. O Becker M. A. & Seegmiller J E. unpublished data, 1971

20. Raivio, K. O Kekomäki, M. P & Mäenpää, P H. Fructose-induced depletion of liver adenine nucleotides dose-dependence and specificity of the fructose effect. Biochem. Pharmacol. 18 2615, 1969

1 Ronca Testoni, S Raggi, A. & Ronca, G Muscle AMP aminohydrolase. III A comparative study on the regulatory properties of skeletal muscle enzyme from various sources. Biochim. biophys. Acta 198 101 1970

22. Sahebjami, H. & Scalettar R. Effects of fructose infusion on lactate and uric acid metabolism. Lancet I 366, 1971.

23. Sirpe, F Della Corte, E., Bonetti, E Abbondanza, A., Abbati, A. & d Stefano, F Fructose-induced hyperuricaemia. Lancet II 1310, 1970.

4 Woods, H. F Eggleston, L. V & Krebs, H. A. *The cause of hepatic accumulation of fructose 1-phosphate on fructose loading.* Biochem. J 119 501 1970.

5. Yu, T F Sirota, J H Berger L., Halpern, M. & Gutman, A. B. Effect of sodium lactate infusion on urate clearance in man. Proc Soc. exp Biol. Med. 96 809, 1957

Acta med. scand. Suppl. 54., pp 115—118

FRUCTOSE AND LIVER PROTEIN SYNTHESIS

Pekka H Mäenpää

From the Department f Medical Chemistry U iversity of Helsinki, 00170 H ls ki 17 F nla d

Abstract Intravenous dministration of 5 mmoles per kg of D-fructose to rats causes transient but strong inhibition of incorporation of [14]C leucine into liver protein. The inhibition is maximal 2 to 8 minutes after the fructose injection and is practically over 25 minutes later. The inhibition of [14]C-leucine incorporation is closely correlated with the depletion of denosine triphosphate (7). Liver polysome profiles are essentially unaltered 5 minutes fter the administration of fructose, but t 15 and 30 minutes gradual and extensive disaggregation f large polysomes is observed. Changes in the hepatic levels of free magnesium are thought to be involved in the alterations of the polysome profiles after fructose administration.

As has been shown by us (7 9) and by others (1 4 13), intravenous or intraperitoneal administration of large doses of fructose rapidly depletes liver adenosine triphosphate in laboratory animals and man. This process probably interferes with most of the liver functions. This report will discuss its effects on liver protein synthesis. It will show that both amino acid incorporation and the size distribution of the hepatic ribosome population are changed after a single intravenous injection of fructose.

MATERIAL AND METHODS

The test animals were female albino rats weighing pproximately 0.2 kg. They were fasted over night prior to the experiments. Incorporation of amino acid into liver protein was examined by giving an intravenous 2-minute pulse of 2 μCi f [14]C DL-leucine (specific activity 53.2 mCi/mmole) at various times relative t an intravenous injection of 5 mmoles per kg of D-fructose. Liver samples were rapidly frozen t the times stated by the rapid-freezing technique and the radioactivity in hot trichloroacetic acid treated protein was determined as described previously (7).

Liver polysome profiles were studied 0, 5, 15, and 30 minutes after injection of fructose. Liver samples were rapidly removed, weighed and homogenized in Tris buffer (25 mM Tris, 25 mM NaCl, and 5 mM $MgCl_2$) containing 0.14 M sucrose and 100 μg/ml f heparin, with seven strokes f Potter Elvehjem type homogenizer Deoxycholate and Triton X 100 were added to final concentration of 1 per cent each, and the tissue was homogenized with two more strokes (8). The resulting 10 per cent liver homogenate was centrifuged for 8 minutes t 30,000 × g. The supernatant (0.2 ml) was layered over 0.5 to 1.5 M

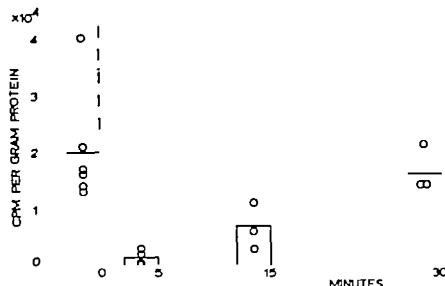


Fig. 1 Effect of intravenous injection of 5 mmoles per kg of D-fructose on incorporation of ^{14}C leucine into liver protein *in vivo* Rats were given an intravenous 3-minute pulse of ^{14}C-leucine at designated times before and after fructose ad ministration. From Mäenpää, Raivio and Kekomäki (7). Copyright 1968 by the American Association for the Advancement of Science.

linear sucrose gradient made in the above buffer plus 40 μg/ml of heparin, and centrifuged for 60 minutes at 192,000 × g_{max} in the SW 50.1 rotor The gradients were analyzed by passing them through a 2-mm Gilford flow cell connected to a Zeiss PMQ II recording spectrophotometer Transmittance at 260 nm was recorded and converted to absorbance units. The absorbance units were multiplied by 5 to correct for the -mm light path.

RESULTS AND DISCUSSION

As shown in Fig 1 incorporation of ^{14}C-leucine into liver protein is strongly inhibited 2 to 5 minutes after fructose administration being less than 10 per cent of the control value The fructose effect is brief and by 30 minutes the value is again the same as in the controls. Comparison of the time courses of the changes in ATP levels and in ^{14}C-leucine incorporation indicates a close correlation between these two variables (7). The exact mechanism by which fructose influences amino acid incorporation is unknown, but the parallel alterations in ATP and in incorporation of the label suggest that it may be due to blocked aminoacylation. In some other test systems the levels of cellular ATP and incorporation of radioactive amino acid into protein show an analogous correlation, although the time course may differ (5 11 12) It should be pointed out that the fructose effect is very rapid.

Fig shows the sedimentation characteristics of hepatic total ribosome population before and at various times after the administration of fructose The dose was identical to that used previously The size distribution of the ribosomes remains essentially unchanged at the time of maximal inhibition of ^{14}C-leucine incorporation. However at 15 and at 30 minutes, a significant gradual disaggregation of the large polysomes into monosomes is observed which suggests that the ribosomes are incapable of initiating a new cycle of peptide synthesis. Although the precise relationship between fructose administration and altered hepatic polysome profiles remains to be determined,

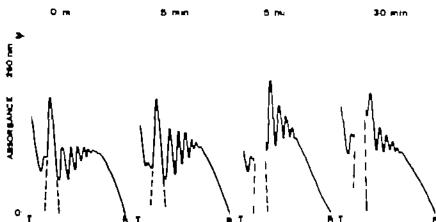


Fig. 2. Liver polysome profiles before and at various times after injection of fructose as in Fig. 1. Polysomes were prepared and analyzed as described in the text. Direction of sedimentation in the sucrose gradients is from left to right and location of the monosome peak in each gradient is indicated by interrupted lines.

changes in free magnesium levels may be involved. A large part of the magnesium present in the cell is normally bound to ATP. After fructose injection when liver ATP is depleted, part of the magnesium may be released into the blood. In this connection it is interesting to note that in a patient with hereditary intolerance to fructose serum magnesium levels were increased after fructose administration (6). When the rebound of liver ATP takes place, the newly formed ATP may lower the free magnesium level still further by binding part of it. Thus, the situation after fructose administration may bear a resemblance to that demonstrated *in vitro* when the so-called runoff ribosomes are released from polyribosomes by incubation under conditions of amino acid incorporation with less than 7 mM Mg^{2+} (2, 3). Here, the total ribosome population was studied. Administration of fructose may also influence the distribution of ribosomes between free and membrane-bound forms, since magnesium is thought to play an important role in the ribosome-membrane interaction (10). A recent ultrastructural study supports this view (14).

These data indicate that parenteral administration of large doses of fructose to rats has multiple effects on liver protein synthesis. Since fructose has been shown to lower the hepatic ATP level in man also (1) caution is called for when intravenous fructose is used for various reasons in man.

ACKNOWLEDGEMENTS

This study was supported by the Signe and Ane Gyllenberg Foundation, Finland.

REFERENCES

1. Bode, Ch., Schumacher H., Goebell, H., Zelder O & Petzel, H. Fructose induced depletion of liver adenine nucleotides in man. Horm metab. Res. 3 289 1971.
2. Bont, W S., Rezelman, G Meisner I & Bloemendal, H. Studies on cytoplasmic ribonucleic acid from rat liver VIII. Stability

of polyribosomes from normal and regenerating rat liver Arch. Biochem. Biophys 119 36, 1967

3. Bont, W S., de Vries, M., Geels, J & Huizinga, A. The effect of the Mg^{2+} concentration on the sedimentation coefficient of ribosomes from rat liver Eur J Biochem. 19 11, 1971
4. Burch, H. B Lowry O H., Meinhardt, L., Max, Jr P & Chyu, K.-J Effect of fructose, dihydroxyacetone glycerol, and glucose on metabolites and related compounds in liver and kidney J biol. Chem. 45 2092, 1970
5. Freudenberg, H. & Mager J Studies on the mechanism of the inhibition of protein synthesis induced by intracellular ATP depletion. Biochim. biophys. Acta 232 537 1971.
6. Levin, B Oberholzer V G Snodgrass, G J A. I Stimmler L. & Wilmers M. J Fructosaemia. An inborn error of fructose metabolism. Arch. Dis. Child. 38 220 1963.
7. Mäenpää, P H Raivio, K. O & Kekomäki, M. P Liver adenine nucleotides Fructose-induced depletion and its effect on protein synthesis. Science 161 1253, 1968.
8. Palmiter R. D Interaction of estrogen, progesterone and testosterone in the regulation of protein synthesis in chick oviduct. Biochemistry 10 4399 1971
9. Raivio K. O Kekomäki, M. P & Mäenpää, P H. Depletion of liver adenine nucleotides induced by D-fructose. Dose-dependence and specificity of the fructose effect. Biochem. Pharmacol. 18 2615 1969
10. Sabatini, D D., Tashiro Y & Palade, G E. On the attachment of ribosomes to microsomal membranes. J mol. Biol. 19 503, 1966.
11. Van Venrooij, W J W Henshaw E. C. & Hirsch, C. A.. Effects of deprival of glucose or individual amino acids on polyribosome distribution and rate of protein synthesis in cultured mammalian cells Biochim. biophys. Acta 259 127 1972.
12. Villa-Trevino, S., Shull, K. H. & Farber E. The role of adenosine triphosphate deficiency in ethionine-induced inhibition of protein synthesis J biol. Chem. 238 1757 1963.
13. Woods H. F Eggleston, L. V & Krebs, H. A. The cause of hepatic accumulation of fructose 1 phosphate on fructose loading. Biochem. J 119 501 1970.
14. Yu, D T & Phillips, M. J.. Hepatic ultrastructural changes in acute fructose overload. J Ultrastructure Res. 36 222, 1971

Acta med. scand Suppl. 542, pp 119—129

METABOLISM OF FRUCTOSE AND GLYCERALDEHYDE IN THE ISOLATED PERFUSED PIG LIVER

L. Sestoft, S. Damgaard, N Tygstrup and F Lundquist

From the Department f Biochemistry A, Department of Medicin A, Department f Surgery C, University of Copenhagen, Department f Clinical Physiology M nicipal Hospital, Copenhagen, and Department f Biochemistry University f Odense

Abstract. Isolated pig livers were perfused under near physiological conditions, i.e. use of pigs blood, perfusion via both *arterial and portal system*, and constant pH (7 4). Fructose uptake and the production of a number of metabolites were measured in relation to the fructose concentration in the perfusion medium. A Michaelis type kinetics was found with V_m about 3 μmoles/g liver x min and K_m bout 8 mM. Fructose-1 phosphate was observed to accumulate as consequence f the inhibition of the aldolase step, presumably by glyceraldehyde, AMP and IMP Sorb tol formation is of small importance under our experimental condition.

During continuous fructose infusion the concentration of glyceraldehyde was low (less than 0 5 mM). Therefore the metabolism f glyceral dehyde was studied separately Measurement of the reaction products glycerol and glycerate as function of the glyceraldehyde concentration support the assumption that glyceraldehyde is metabolized by several pathways

Measurement of acid production during metabolism of either fructose or D-glyceraldehyde suggests that considerable accumulation f acids occur in the liver as only minor part of the titration value is accounted for as the organic acids measured, of which lactic acid is the quantitatively most important

Only 20—30 / f the carbon f fructose and glyceraldehyd is accounted for through formation f lactate and glucose. The rest is oxidized or accumulates in some form in the liver

The oxygen consumption during fructose metabolism was increased about 30 , and the lactat /pyruvate ratio showed small, but reproducible changes.

The metabolism of fructose in the liver still presents some problems, which need clarification, *viz.*

1. How does the metabolism of fructose depend on the concentration of fructose in the blood?
2. What is the relative contribution of the various pathways suggested to account for the fate of glyceraldehyde? (cf. Fig. 1).
3. How does fructose (and glyceraldehyde)

The team responsible for this investigation further includes K Tønnesen, F Valla Hansen and K. Winkler

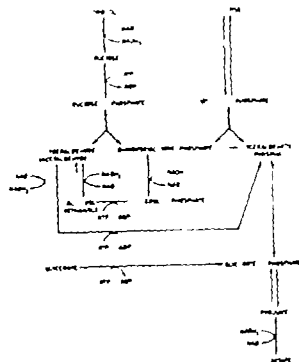


Fig. 1. Metabolic pathways of fructose in the liver.

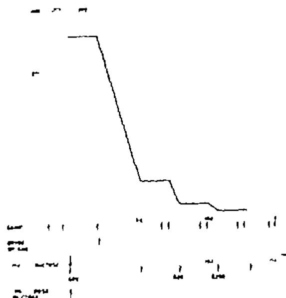


Fig. 2. The calculated time course of an experiment with declining fructose elimination rate. The infusion of fructose was calculated as μmoles/g x min. Periods with constant infusion were 21 minutes. After each steady state period the infusion was stopped for the period necessary to reach the next steady state fructose concentration.

influence the metabolic condition of the liver (redox state, oxygen consumption, phosphorylation potential, etc.)?

Some of these problems were approached by means of perfusion of the isolated pig liver. This preparation was chosen primarily because of the importance of this system in the treatment of hepatic coma by extracorporeal perfusion, but also because the large volume of circulating blood permits the simultaneous determination of a very large number of metabolites.

METHODS

Pig livers were perfused with heparinized pig blood diluted with physiological saline to a hematocrit value of about 18. Perfusion was performed simultaneously through the portal vein and the hepatic artery at physiological pressures. The pH of the circulating medium was kept at 7.4 by titration with $NaHCO_3$ as considerable amounts of acid are produced in these experiments. The anoxic period involved in establishing the artificial circulation was in general 3—4 min at 33°C. The rate of perfusion was kept constant and the pressure in both arterial and portal system continuously recorded. The blood was equilibrated with 95 % O_2 — 5 % CO_2. Details of the perfusion system have been published (11).

The material comprises 11 experiments. Some of the physiological parameters are listed in Table I. The experiments were started after an equilibration period of about 60 min, including a period in which pressure and temperature were unchanged for at least 20 min, and no acid production was recorded by the titrator. In order to obtain steady state conditions the substrates (fructose or glyceraldehyde) were infused at constant rates for about 20 min. The infusion rate necessary to establish a desired concentration in the system was calculated from preceeding experiments, in which a single large dose was given and the elimination followed until the substrate was completely removed. Fig. shows an experimental plan in which the theoretical fructose concentrations were calculated from such an experiment with a single dose of fructose. Fig. 3 shows the levels actually obtained.

Table I. *Physiological parameters of the present experiments (mean ± SD (n = 11))*

Liver weight (kg)	1.191 ± 0.077	Arterial pressure (mm Hg)	123 ± 35
Blood volume (l)	3.56 ± 0.59	Portal pressure (mm Hg)	12.9 ± 4.4
Total flow (l/min)	1.22 ± 0.21	Transsinusoidal resistance (mm Hg/l × min)	11.0 ± 4.2
Arterial flow (l/min)	0.17 ± 0.14	Bile flow (µl/min)	360 ± 1 0

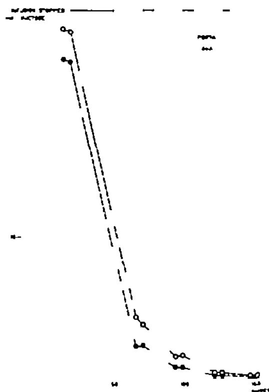


Fig. 3. The actual fructose concentrations obtained in the experiment referred to in Fig. 2.

RESULTS

Initial steps in fructose elimination

Fig. 4 shows the rate of fructose elimination as a function of the fructose concentration in the portal blood (affluent blood). The elimination rate was calculated both from the simultaneous concentration difference over the liver and from the change in concentration with time of the pool of circulating blood.

Three processes might be considered as candidates for the rate limiting step in fructose uptake by the liver 1) transport of fructose across the cellular membrane, 2) reduction to sorbitol and 3) phosphorylation to fructose-1 phosphate (or 6-phosphate).

The possibility that some transport process is rate limiting does not appear likely as our limited biopsy material suggests that equilibrium is established between tissue and circulating medium.

The formation of sorbitol, which seems to be a dead end in fructose metabolism is of small importance under our experimental conditions (Table II). The concentration of sorbitol in the medium is about 3—4 / of the fructose concentration under steady state conditions indicating equilibrium during the steady periods (2), but not in the period when the fructose concentration falls precipitously

Phosphorylation to fructose-1-phosphate has repeatedly been described in other species (3 6 13). Also in the pig a considerable accumulation occurs as seen in Table III, indicating that ketohexokinase is of importance also in the pig. The concentration of fructose-6-phosphate was not significantly increased in the biopsies. When our elimination curve was converted to a Lineweaver Burk plot a fair approximation to Michaelis kinetics was observed. The kinetic parameters for the elimination are given in Table IV together with results on crude preparations from pig liver A 10 times higher K_m value was found for the perfused liver However the measurements of K_m reported in the lite-

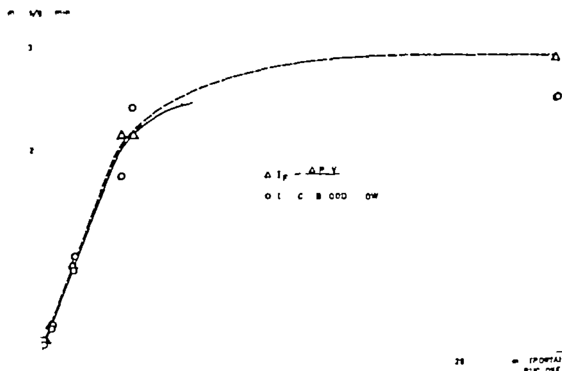


Fig 4 The elimination rate of fructose versus the fructose concentration in the affluent medium calculated from the experiment referred to in Fig. _ Two methods of calculation have been employed Δ = Infusion rate corrected for simultaneous accumulation of substrate in the medium, 0 = concentration difference between affluent and efluent medium times total flow

Table II. *Sorbitol concentration in the affluent blood during increasing fructose elimination At 90 minutes the fructose infusion was stopped*

Time (min)	Portal concentrations (mM) Fructose	Sorbitol	Sorbitol/Fructose
—5	0 00	0 0[illegible]3	0 00
60	4 63	0 065	0 01
[illegible]5	10 [illegible]4	0 [illegible]70	0 03
90	14 1	0 5 0	0 04
110	3 [illegible]0	0 330	0 12

rature (0.5 mM see ref. 1 8) as well as our own measurements were made in the virtual absence of ADP The presence in the liver of this substance,which is known to modify the ketohexokinase reaction, may be the explanation for the high apparent K_m found *in vivo* Interference from hexokinase at high fructose concentrations appears less likely but cannot be excluded.

The aldolase step

The splitting of fructose-1 phosphate into glyceraldehyde and dihydroxyacetone phosphate is apparently a bottleneck in the metabolism of fructose in liver tissues, since fructose-1 phosphate may accumulate in very large quantities. This step was followed through measurement of glyceraldehyde Fig 5 shows a characteristic feature of the experiments. Immediately after fructose infusion the glyceraldehyde concentration increases up to about 1 mM and then drops off

Table III. *Concentrations of fructose 1 phosphate in the liver biopsies The figure below the bottom line is a mean value from 3 pigs not given fructose*

Fructose-1 P (μmoles/g liver)	Fructose (mM, Cava)
1.1	0.10
1.3	0 19
5.1	2.5
6.3	2 0
3.6	2.6
0 7 (n = 3)	0 0

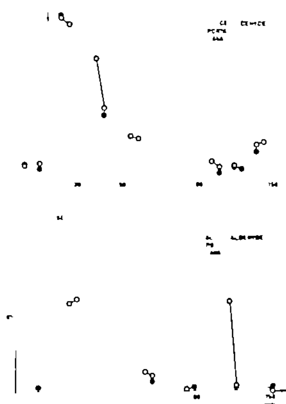


Fig 5 Concentration of D-glyceraldehyde during stepwise reduction of fructose concentration from 25 mM The lower part of the figure is derived from the experiment shown in Figs 2, 3, 4

rapidly while the fructose concentration is still high. After nearly complete removal of the fructose, the liver may liberate glyceraldehyde again as seen in Fig 6. The obvious interpretation of these results is that the aldolase step is strongly regulated, as was in fact shown by Woods et al. (13) who observed inhibition by D-glyceraldehyde, AMP and IMP Initially after administration of a large dose of fructose the concentration of the three substances mentioned will be low and accordingly the aldolase will function at full capacity resulting in an outburst of D-glyceraldehyde This in itself together with the increasing concentrations of AMP and IMP inhibits the cleavage of fructose-1-phosphate, which accordingly accumulates.

When the supply of free fructose is exhausted the fructose-1-phosphate accumulated will again give rise to glyceraldehyde, as AMP and IMP are rapidly further metabolized (presumably to allantoin) This may explain the secondary bursts of glyceraldehyde described above.

A consequence of these findings is that under steady state conditions the concentration (found to be less than 0.5 mM) and the output of glyceraldehyde will be low and apparently dependent on the previous history of the liver We therefore performed some

Table IV *The elimination kinetics of fructose in the isolated perfused pig liver compared to the kinetic constants of the pig liver ketohexokinase (Mean values ± S.E.M.) The results for ketohexokinase were obtained on high speed supernatant directly (V_m) or after acid treatment (K_m) to remove sorbitol dehydrogenase*

	K_m (mM)	Maximum elimination rat (μmoles/g × min)
Perfused pig liver	5 9 ± 0 4 (n = 4)	2.9 ± 0.2 (n = 4)
Pig liver ketohex kinase	0.5 (n = 2)	1.9 ± 0.4 (n = 4)

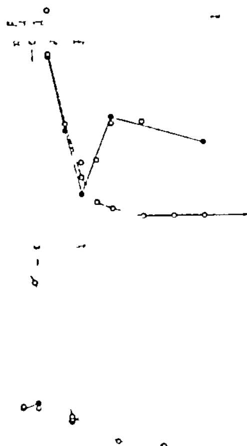


Fig. 6. Release f D-glyceraldehyde coincident with cessation of fructose elimination.

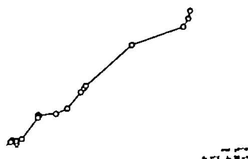


Fig. 7 Elimination rate of D-glyceraldehyde as function of the concentration in the affluent medium.

experiments with infusion of D-glyceraldehyde in the perfused pig liver in order to examine the fate of glyceraldehyde at higher concentrations.

D-glyceraldehyde metabolism

Several metabolic pathways may be open to glyceraldehyde (see Fig 1) oxidation by means of NAD-requiring aldehyde dehydrogenase to glycerate, reduction to glycerol by NAD or NADP requiring enzymes, phosphorylation to glyceraldehyde-3-phosphate by triokinase and condensation with dihydroxy acetone phosphate to fructose-1-phosphate. Fig 7 shows the rate of glyceraldehyde removal as a function of the concentration in the circulating medium. It is striking that the removal of glyceraldehyde is very slow (0.2—1 μmoles/g min) at concentrations corresponding to those in the steady state fructose experiments. The various plateaus may correspond to individual processes, but it should be mentioned that the curve cannot be constructed simply by addition of a series of Michaelis curves. However Fig 8, in which the concentrations of lactate, glycerate and glycerol at different glyceraldehyde concentrations are given, suggests that new proces-

Table V Kinetic constants of some pig liver enzymes involved in D-glyceraldehyde metabolism (Mean ± S.E.M.) D-Gald D-glyceraldehyde D-Ga D-glycerate

Enzyme	Coenzyme	Substrat	K_m (mM)	V_{max} (U/g)
Alcohol d hydrogenase	NADH	D-Gald	18 ± 2	14 ± 3 (n = 4)
Alcohol d hydrogenase	NADPH	D-Gald	5 6 (n = ?)	4.3
Aldehyde d hydrogenase	NAD	D-Gald	0 16 (n = ?)	0 64 ± 0 07 (n = 4)
Glycerate kinase	ATP	D-G	—	0 17 ± 0 08 (n = 8)

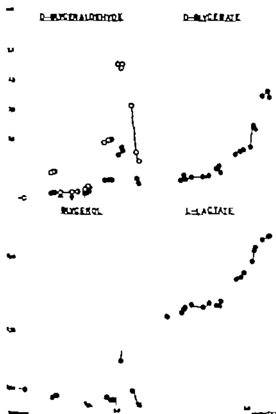


Fig. 8. Concentrations of D-glycerate, glycerol and L-lactate in the effluent medium in an experiment in which D-glyceraldehyde was infused to maintain different concentration levels in the affluent medium.

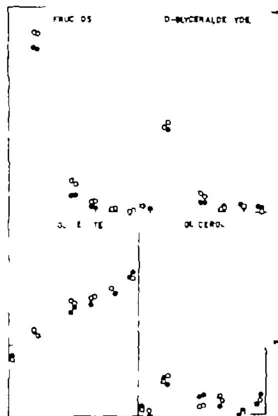


Fig. 9 Concentrations of D-glyceraldehyde glycerol and D-glycerat in the affluent and effluent media in an experiment in which fructose was infused to maintain different concentration levels in the affluent medium.

ses prevail as the concentration of aldehyde is increased, although the figures can only be taken as rough indicators of the rate of the primary processes, as these substances may be further metabolized. The lack of glycerol formation except at very high concentrations of D-glyceraldehyde is in agreement with the high apparent K_m for ADH (both NADH and NADPH requiring) found with crude preparations from pig liver (Table V) It is noteworthy that the activity of glycerate kinase is very low (Table V) in the pig in contrast to some other species (10). In agreement with this finding an accumulation of glycerate is always observed whether glyceraldehyde or fructose is used as substrate in the perfused pig liver (cf. Figs. 8 and 9).

Infusion of glycerol and glycerate at a concentration of 3 mM did not give rise to any production of pyruvate and lactate. It therefore seems likely that triokinase is responsible for the pyruvate formation from glycer aldehyde under our experimental conditions. However virtually no lactate (or glucose) production was found at glyceraldehyde concentrations corresponding to those observed during steady state fructose elimination.

The major metabolites given off from the liver metabolizing fructose or glyceraldehyde are lactate and glucose. In Fig. 10 the output

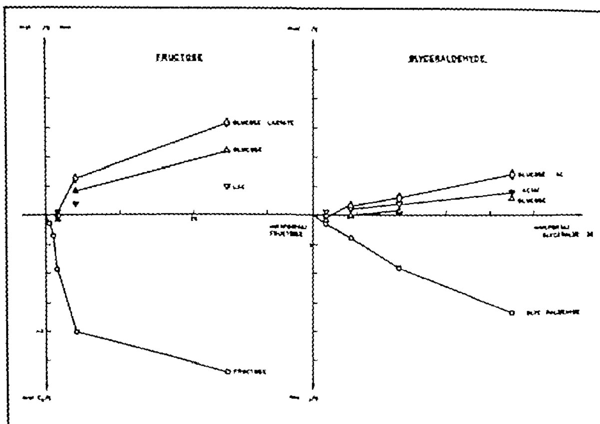


Fig. 10 Output of glucose and L-lactate and uptake of fructose and D-glyceraldehyde respectively calculated as C_3 units in dependence of the concentration of substrate in the affluent medium

of these substances is compared to the uptake of fructose and glyceraldehyde respectively The glucose formation relative to lactate is much higher with fructose than with glyceraldehyde. With both substrates only about *0 per cent of the substances infused is accounted for as glucose + lactate. This of course means that the remaining part is either metabolized complet to CO_2 and water or deposited in f (as glycogen, fat or phosphory ...lates). Even if it is assumed the oxygen uptake is used for the c the added substrate a re must be deposited in ly the CO_2 producti d with sufficient a quanti tative

Acid production. Fig. 11 shows the acid production during experiments with fructose and glyceraldehyde. The titrations are compared with the uptake of the substrate in each of the experimental periods. A simple connection between these two parameters is not to be expected, but some information may be obtained. For instance, in the first period of the fructose experiment the fructose uptake is nearly equal to the hydrogen ion output. The organic acids measured include lactate pyruvate, glycerate, acetoacetate, β-hydroxybutyrate, citrate, malate, and glutamate. No change in the concentration of ketone bodies v ved, but malate and citrate showed a ant increase (about 3 times). How total organic acids measured account only 40—50 per cent of the titration ...gesting intracellular accumulation g in with phosphoryla

...ycera eriment there is ...ubstrate ...ations a striking

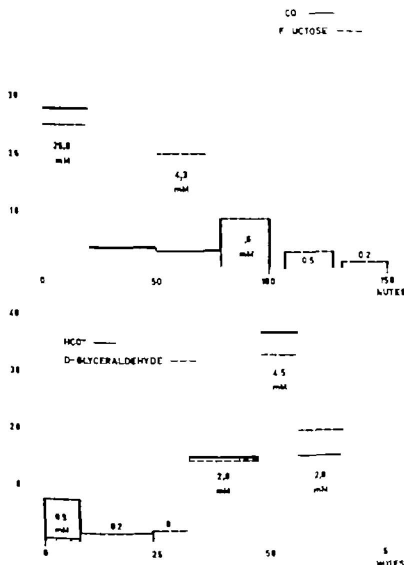


Fig 11 Acid production and elimination of fructose and D-glyceraldehyd respectively. The figures inserted in the columns represent average concentrations in the affluent medium.

similarity in the uptake of substrate and the output of acid equivalents. Again the organic acids (pyruvate lactate, glycerate) account for only part of th titration (about 30 per cent. The rest seems to be intracellular accumulation of acids.

Metabolic parameters

The oxygen uptake has consistently been observed to increase when fructose is infused both in man (12) in the cat (7), and in the present experiments. Hassinen (4) however in perfused rat liver found a (transient?) decrease of oxygen uptake. In the pig liver an average increase of 30 per cent was found. At least two factors would tend to increase respiration, *viz.* an increased ADP concentration, which is believed to result from the fast phosphorylation of fructose, and the decreased ATP concentration, which will activate pyruvate dehydrogenase (9) and there-

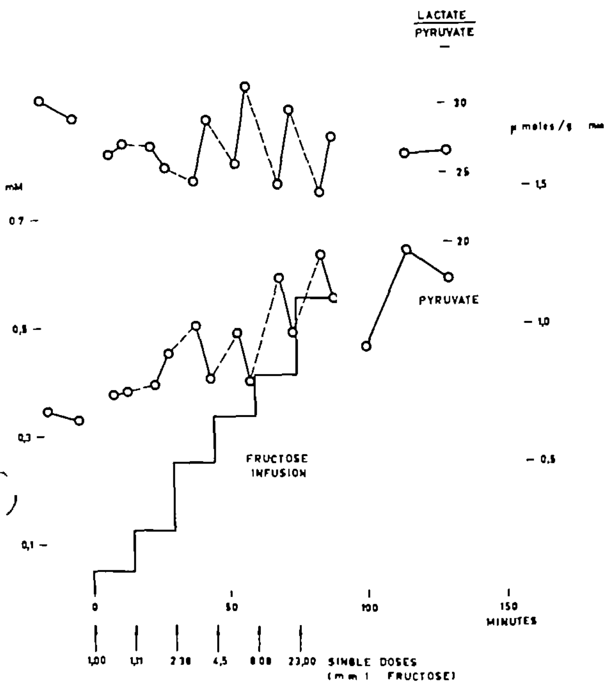


Fig. 12. Changes in lactate-pyruvate ratio during stepwise increasing elimination rate of fructose. Single doses of fructose were administered as indicated, and the concentration levels kept constant with stepwise increasing fructose infusion.

fore increase the input of pyruvate in the TCA cycle as pyruvate is present in abundance under these conditions. Attempts to measure the adenosine phosphates in biopsies, were unfortunately unsuccessful.

The cytoplasmic redox state as measured by the lactate/pyruvate ratio showed small but characteristic changes when the fructose load is altered as seen in Fig 12. At each increase in the fructose infusion rate a transient decrease in the ratio was observed caused by an increased pyruvate concentra-

tion. The increased pyruvate level may be caused by increased activity of pyruvate kinase, which is known to be activated by fructose-1 phosphate (3). On the other hand the temporary decrease in ATP assumed to accompany fructose phosphorylation would release the inhibition of pyruvate dehydrogenase and thereby cause a fall in the pyruvate concentration. The biphasic curve might be caused by the consecutive action of the two mechanisms. (The changes in lactate/pyruvate ratio are small both with fructose and with glyceraldehyde)

CONCLUSIONS

Fructose is eliminated by the isolated pig liver in a concentration dependent way resembling Michaelis kinetics. The maximal uptake was about 3 μmoles per g liver per min. Half this rate was observed at a concentration about 5 mM (K_m).

The experimental findings are in agreement with a strong regulation of the aldolase reaction, resulting in accumulation of fructose-1-phosphate in the liver

Experiments with glyceraldehyde alone indicate that several processes are involved in the metabolism of this substance. Glycerate and glycerol ware identified as intermediates. The relation between concentration and rate of metabolism of glyceraldehyde is a complicated one.

The output of glucose and lactate when fructose or D-glyceraldehyde is infused accounts for about 20 per cent of the elimination, but the production of acid equivalents is considerably higher suggesting intracellular accumulation of acids.

The oxygen uptake during fructose metabolism was increased about 30 per cent.

REFERENCES

1 Adelman, R C Ballard, F J & Weinhouse, S Purification and properties of the rat li er fructokinase. J biol. Chem. 242 3360, 196

2 Blakly R L The metabolism and antiketogenic effects of sorbitol, sorbitol dehydrogenase Biochem. J 49 257 1951.

3 Eggleston, L V & Woods, H. F Activation of liver pyruvate kinase by fructose 1-phosphate FEBS Letters 6 43, 1970

4 Hassinen, I & Ylikahri, R. H. Absorption spectrophotometry f perfused rat liver ppl ed to fructose-induced inhibition of respiration Biochem biophys Res. Comm. 38 1091 1970

5 Hers, H G & Kusaka, T Le metabolisme du fructose-1 phosphate dans l foie. Biochim. bi phys Acta 11 427 1953

6 Kjerulf-Jensen, K The phosphate esters formed in the liver tissue f rats and rabbits during assimilation of hexoses and glycerol Acta physiol. scand 4 249 1942.

Lundsgaard, E Nielsen, N A & Orskov S. L. The carbohydrate metabolism of the isolated cat liver Scand. Arch. Physiol 73 296, 1936

8 Parks, R E Ben-Gershom, E. & Lardy H. A Liver fructokinase J biol. Chem. 227 231 1957

9 Soling, H D & Bernhard, G Interconversion of inactive to active pyruvate dehydrogenase in rat liver after fructose pplication in vivo FEBS Letters 13 201, 1971

10 Thieden, H. I. D Grunnet, N., Damgaard, S E & Sestoft, L. Effect of fructose and glyceraldehyde on thanol metabolism in human and rat liver Eur J Biochem (in press).

11. Tygstrup N Funding, J Juul Nielsen, J Keiding, S Koudahl, G Ramsøe, K. & Winkler K. The function of the isolated perfused and the in vivo pig liver Scand. J Gastroenterol. Suppl. 9 131 1971.

12. Tygstrup, N., Winkler K. & Lundquist, F.. The mechanism of the fructose effect on the ethanol metabolism of the human liver J clin. Invest. 44 817 1965

13 Woods, H. F Eggleston, L. V & Krebs, H. A The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. Biochem. J 119 501, 1970.

Acta med. scand. Suppl. 54., pp 131—140

THE INTERRELATIONSHIP BETWEEN FRUCTOSE AND ETHANOL METABOLISM IN THE ISOLATED PERFUSED PIG LIVER

S. E. Damgaard, L. Sestoft, F Lundquist and N Tygstrup

From the Department of Biochemistry A, Department of Medici e A, Department of Surgery C University of Copenhagen, Department of Clinical Physiology Municipal Hospital, Copenhagen and Department of Biochemistry University f Odense

Abstract In the isolated perfused pig liver the rate of ethanol elimination is about 0.7 µmol/min/g. The acetate produced from ethanol is metabolized at a rate of about 0.5 µmol/min/g. Metabolism of ethanol does not affect oxygen or acetate uptake in this preparation. Alcohol dehydrogenase activity in pig liver is about 2 µmol/min/g and is assumed to account for practically all of the ethanol oxidation observed under the experi mental conditions. Fructose increases the ethanol elimination rate to more than twice the control value. Oxygen consumption is similarly increased during this stimulation. A relationship between the fructose elimination rate and the stimulation of ethanol elimination is obtained. Pyruvate and D-glyceraldehyde stimulate ethanol elimination, but in both cases the increase is less than 50 % of the increase observed with fructose. Uncoupling of oxidative phosphorylation from respiration by dinitrophenol does not affect the basal ethanol elimination rate, but it decreases the fructose effect on ethanol oxidation to less than half. Accumulation of reduced metabolites during stimulation of ethanol elimination by fructose and D-glyceraldehyd only accounts for minor part of this effect From these bservations it is assumed that an energy dependent cyclic process (the 'malic enzyme shuttle') converts th reduction equivalents produced in th alcohol dehydrogenase reaction as NADH to NADPH.

The team responsible for this investigation further includes G Kondahl, K. Tønnesen, F Valle Hansen and K. Winkler

Fructose has been found to accelerate ethanol metabolism in man, in dogs, and in rats (6, 10 11 13 14, 16) In other studies small or no effects have been noted (5).

The interaction of fructose and ethanol metabolism may throw light upon important metabolic regulations in the liver The isolated perfused pig liver is a suitable ex perimental model for the study of this problem, because it has greater metabolic resem blances with the human liver than rat liver has, because it is free from the regulatory mechanisms which the body exerts on the liver *in situ* and because it permits ex perimental manipulations which are not feasible in man.

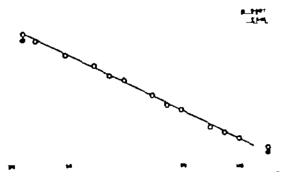


Fig. 1 The concentration of ethanol in afflüent (O) and effluent blood (●) during pig liver perfusion with continuous infusion of ethanol (0.46 mmoles/min).

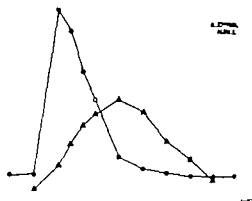


Fig 2 The elimination of a single dose of ethanol (O) and the production and subsequent elimination of acetate (△) in the isolated perfused pig liver

The technique of isolated pig liver perfusion has been described in the previous paper together with the fructose metabolism of that preparation (12).

ETHANOL METABOLISM IN THE ISOLATED PIG LIVER

Fig. 1 shows the disappearence of ethanol from the medium following continuous infusion of 0.45 mmoles/min of ethanol for 40 minutes. The elimination rate of ethanol is constant from concentrations of about 10 mmoles/l to about 1 mmole/l, as reflected by linear fall in concentration and a constant concentration difference between afffluent and effluent blood.

Fig 2 shows the elimination curve after a single injection of 21 mmoles of ethanol. There is a small amount of ethanol in the medium before the injection. The concentration of acetate in the medium rises almost linearly during the first period of constant elimination rate of ethanol, and falls after the ethanol has been eliminated. The elimination rate of ethanol is calculated from the slope of the elimination curve, the volume of perfusate, determined by ^{131}I labelled albumin, and the hematocrit. The loss of ethanol from the system by evaporation in the bubble oxygenator was insignificant.

The conversion of acetate by the liver during elimination of ethanol is assessed as the difference between the calculated production of acetate from ethanol and the observed accumulation of acetate in the medium. When no production from ethanol takes place the elimination of acetate is determined as for ethanol.

Table I shows the data for ethanol and acetate elimination in the isolated perfused pig liver The elimination of ethanol was about one third of the value observed for the human liver in vivo (9). About 60 per cent of the acetate produced from ethanol is activated in the isolated liver against less than 25 per cent in the intact human liver In a liver studied without a simultaneous break-down of ethanol, a similar elimination of acetate was seen. Therefore elimination of ethanol apparently does not inhibit the activation of acetate.

The oxygen uptake of the isolated liver appears to be unaffected by ethanol. About 60 per cent of the oxygen uptake can be accounted for by oxidation of ethanol to acetate Furthermore the table shows that

Table I. *Ethanol metabolism of the isolated perfused pig liver*

Period	μmol/min/g ± S.E.M. (number of experiments) Ethanol	Oxygen	Acetate
No ethanol	—	1.23 ± 0.05 (8)	0.46 (1)
Ethanol	0.71 ± 0.05 (6)	1.19 ± 0.17 (6)	0.47 ± 0.04 (4)
Supernatant + ethanol	2.14 ± 0.19 (6)		

the activity of alcohol dehydrogenase in the supernatant fraction of pig liver homogenate is considerably greater than the elimination rate in the perfused organ. The K_m of the enzyme for ethanol is about 1.5 mmoles/l at pH 8.8.

At an ethanol concentration of 30 mmoles/l the ethanol elimination rate was the same. This indicates that no ethanol oxidizing system with a K_m much higher than for alcohol dehydrogenase contributes significantly to the observed ethanol elimination rate. This is supported by experiments with pyrazole known to inhibit alcohol dehydrogenase (Table II). While the inhibition of ethanol elimination is almost complete, acetate elimination is not affected. The lactate to pyruvate ratio of the effluent blood is sharply reduced.

EFFECT OF FRUCTOSE AND FRUCTOSE METABOLITES

The effect of fructose on ethanol elimination is shown in the experiment of Fig 3, in which a constant infusion of 1.52 mmoles/min of ethanol and a stepwise infusion of fructose, 0.00 0.26 0.86, 1.71, and 2.56 mmoles/min, was given. The increase in ethanol elimination is reflected by a reduced accumulation of ethanol in the medium, as well as by an increase in the concentration difference between affluent and effluent blood The effect appears to depend on the dose of fructose given In Fig 4 the relation between the elimination rate of ethanol and the concentration of fructose in the medium is shown together with the curve of the fructose concentration–elimination relationship The latter curve is identical with curves obtained in the absence of ethanol (12) With the highest concentration of fructose used, the elimination rate of ethanol is more than doubled, and we therefore can confirm the fructose-ethanol effect in this preparation.

Table II. *The effect of pyrazole (8 mmoles/litre) on ethanol metabolism of the isolated perfused pig liver*

Experiment	Period	μmol/min/g Ethanol	Acetate	Ratios L/P	H/A
84	Control	0.77	0.42	340	5
	Pyrazole	0.15	0.42	30	10
90	Control	0.71	0.41	210	12
	Pyrazole	0.00	0.35	17	6

The ability of fructose to stimulate the oxidation of ethanol has been attributed to its rapid conversion to D-glyceraldehyde or pyruvate. Both substances stimulate the oxidation of ethanol (6, 14, 15, 17). It was therefore of interest to compare their effect with that of fructose in the same preparation.

In two experiments (Table III) pyruvate was infused, and concentrations of pyruvate in the medium of 1.3 and 20 mmoles/l were obtained. It is seen that despite a fairly high uptake of pyruvate by the liver the stimulation of ethanol elimination was moderate, viz. 30—50 per cent.

In two experiments (Table IV) the effect

CONTINUOUS INFUSION OF ETHANOL

STEPWISE INFUSION OF FRUCTOSE

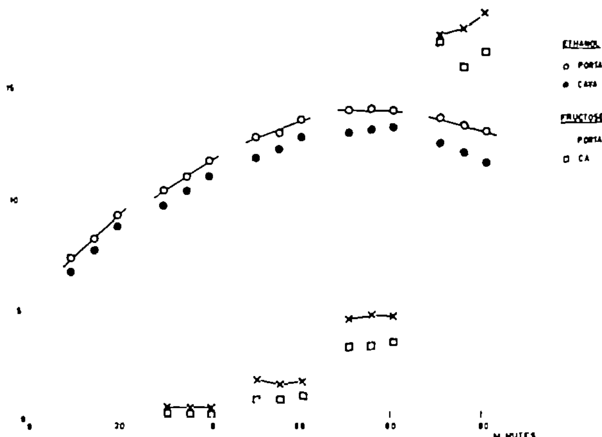


Fig 3 Infusion rates Ethanol 1.52 mmoles/min. Fructose 0.26 0.86 1.71 and 2.56 mmoles/min.

of D-glyceraldehyde infusion was studied. The concentration of D-glyceraldehyde in the medium was 2 and 4 mmoles/l, respectively This is four to eight times higher than that observed during fructose stimulation of ethanol elimination. The ethanol elimination rate is increased, but the stimulation by D-glyceraldehyde is smaller than by fructose although the elimination rates (in μmol/min/g) are similar

Table III *The effect of pyruvate on ethanol metabolism of the isolated perfused pig liver*

		Pyruvate		Ethanol	L/P
Experiment	Period	mean conc. mmoles/ litre	elimination rate μmol/ min/g	elimination rate μmol/ litre	(effluent blood)
105	Control	0 02	0.00	1 00	731
	Pyruvate	1.3	2.0	1 48	87
111	Control	0.03	0 00	1.09	125
	Pyruvate	20.0	3 5	1 29	0.48

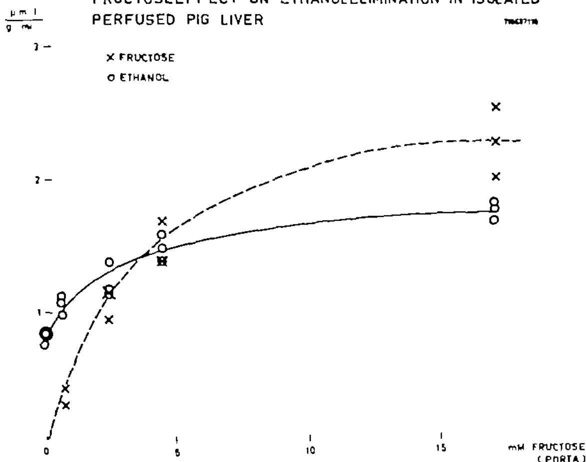


Fig. 4. The data are derived from the same experiment as shown in Fig. 3.

PRODUCTION OF REDUCED METABOLITES DURING THE 'FRUCTOSE EFFECT'

The rate of ethanol oxidation is considered to be determined by the rate of reoxidation of cytoplasmic NADH formed in the alcohol dehydrogenase catalyzed reaction, and not by the alcohol dehydrogenase activity itself (2, 3) Stimulation of ethanol oxidation may therefore be the result of an increased re-

Table IV *The effect of D-glyceraldehyde on ethanol metabolism of the isolated perfused pig liver*

Experiment	Period	D-glyceraldehyde mean conc. mmoles/litre	D-glyceraldehyde elimination rate µmol/min/g	Ethanol elimination rate µmol/min/g	L/P (effluent blood)
107	Control	0 2	0 07	0 60	402
	D-GALD	2.0	1.42	0 79	410
113	Control	0 05	0 00	1.32	336
	D-GALD	4.0	2.87	1.65	400

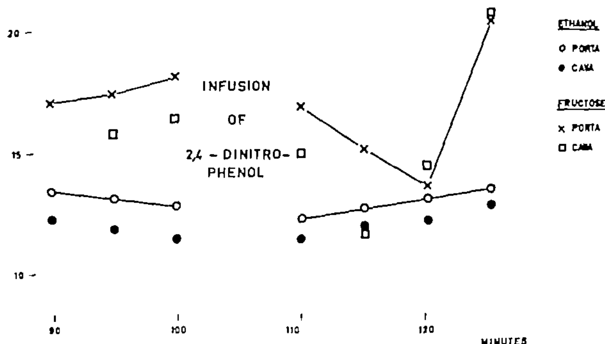


Fig 6 Infusion rates Ethanol 1 52 mmoles/min. Fructose 2 56 mmoles/min. 2.5 mmoles of 2,4 dinitrophenole was infused in the interval from 100 to 110 minutes.

effects suggest that 60 per cent of fructose stimulation of ethanol oxidation depends on the presence of intramitochondrial ATP since DNP action will deprive the mitochondrial matrix before the cytosol of the high energy phosphate.

In rat liver the stimulation of ethanol oxidation by D-glyceraldehyde has been observed (14) If the mechanism is a mere reduction of D-glyceraldehyde to glycerol this effect should be independent of ATP (4, 7). However addition of rotenone abolished the stimulation completely although oxygen consumption in both controls and stimulated assays were only inhibited 50 per cent (15). In isolated hepatocytes pyruvate stimulation of ethanol oxidation was also completely inhibited by rotenone (1)

MALIC ENZYME SHUTTLE

The common need for mitochondrial ATP by these three effects (pyruvate, D-glyceraldehyde, and fructose) indicates a common stimulatory mechanism. Speculations about this

Table VII. *The stimulation of ethanol elimination by fructose and the effect of 2 4-dinitrophenol on this stimulation*

Period	Ethanol	µmol/min/g Oxygen	Fructose	Ratios L/P	H/A
Control	0 8	0.8	0.0	414	18
Fructose	1.8	1.8	2.5	843	[illegible]
Fructose + DNP	1.2	2.2	3.9	189	[illegible]

MALIC ENZYME SHUTTLE

CYTOPLASM

MITOCHONDRIA

ETHANOL
MALATE
NAD
NADH
ACETALDEHYDE
O ALOACETATE
GLYCEROL
CO
NADP
NADPH
P RUV TE
PYRUVATE
FRUCTOSE
D GL CERALDEHYDE
ATP
CO
OXALOACET TE
C TR TE
SPARTATE
GLUTAMATE

Fig. 7 The reactions shown are thought to be responsible for the energy dependent part of the fructose induced stimulation of ethanol elimination.

mechanism have led to the proposal of what may be named the malic enzyme shuttle (15) outlined in Fig. 7 At least four enzyme activities are necessary Alcohol dehydrogenase oxidizes ethanol to acetaldehyde, and this process is probably limited by the increase in the NADH to NAD^+ ratio. The malate dehydrogenase reaction produces NAD^+ from NADH in the cytosol from oxaloacetate. The malate formed in this reaction can be converted to pyruvate by malic enzyme if $NADP^+$ is present. Pyruvate is carboxylated intramitochondrially by an energy requiring process and oxaloacetate is formed. Transport of oxaloacetate from the mitochondria to the cytosol is an indirect process, but can proceed via citrate if malate is present or as aspartate if glutamate is present.

The carboxylation of pyruvate depends on the pyruvate concentration in the physiological concentration range (K_m about 0 4 mM) Therefore pyruvate will stimulate the activity of the shuttle.

This shuttle actually converts NADH to NADPH and would soon stop if NADP + was not reformed from NADPH. An obvious way to do this is to form fatty acids. The acetyl-SCoA which is necessary for these processes is formed intramitochondrially from acetate or pyruvate and can be transported to cytoplasm together with oxaloacetate in the form of citrate. Also other processes in the liver utilize NADPH so it can be assumed that besides fatty acid synthesis there is a basal turnover

D-glyceraldehyde stimulation of 'malic enzyme shuttle can now be explained as an increase in NADP formation from NADPH

as a result of D-glyceraldehyde reduction to glycerol by the NADP dependent alcohol dehydrogenase. Fructose stimulation of this cycle is attributed to its very rapid conversion to D-glyceraldehyde and pyruvate which stimulates the 'malic enzyme shuttle' in two different ways and therefore possesses more stimulatory power than either of its two metabolites.

If this explanation of the fructose effect is correct, then a considerable part of the fructose effect on dehydrogenation of ethanol does not lead to an increased mitochondrial oxidation of the NADH produced, but to anabolic processes via the malic enzyme shuttle involving storage of the reduction equivalents in the form of fat or other reduced substances. This model in contrast to earlier explanations of the fructose effect includes the $NADP^+$—NADPH redox system which is known to change during ethanol metabolism (18) even though the NADP dependent alcohol dehydrogenase does not oxidize ethanol. Furthermore the requirement for mitochondrial ATP is explained by this model.

REFERENCES

1. Berry M. N. The action of pyruvate on ethanol oxidation by intact isolated liver cells. Biochem. J. 123 41 P 1971
2. Edwards, J. A. & Evans D. A. P. Ethanol metabolism in subjects possessing typical and atypical liver alcohol dehydrogenase. Clin. Pharm. and Therap. 8 824, 1967
3. Hillbom, M. E. Influence of sorbitol on the rate of ethanol oxidation and on acetaldehyde accumulation in hypo-, hyper- and euthyroid rats. Lif Sci. 9 641 1970.
4. Holzer H. & Schneider S. Zum Mechanismus der Beeinflussung der Alkohol Oxydations in der Leber durch Fructose. Klin. Wschr 33 1006, 1955
5. Johannsmeier K., Redetzki, H. & Pfleiderer G. Zur Frage der Beschleunigung des Blutalkoholabbaus. Klin. Wschr 32 560, 1954.
6. Leloir L. F. & Munoz, J. M. Ethyl alcohol metabolism in animal tissues. Biochem. J 32. 299 1938.
7. Leuthardt, F. & Wolf, H. P. Über der Glycerindehydrase der Leber. Helvet. chim. Acta 37 1732, 1954.
8. Lundquist, F. & Wolthers, H. The influence of fructose on the kinetics of alcohol elimination in man. Acta pharmacol. Toxicol. 14 290 1958.
9. Lundquist, F. Tygstrup, N. Winkler K., Mellemgaard, K. & Munch-Petersen, S. Ethanol metabolism and production of free acetate in the human liver. J. clin. Invest. 41 955, 1962.
10. Pletscher A., Bernstein, A. & Staub, H. Beschleunigung des Alkoholabbaus durch Fructose beim Menschen. Experientia 8 307 1952
11. Pletscher A., Bernstein, A. & Staub, H. Zur Beeinflussung der Umsatzgeschwindigkeit des Alkohols. (I) Die Beschleunigung des oxydativen Alkoholabbaus durch Fructose Hel. physiol. Acta 10 74, 1962.
12. Sestoft, L., Damgaard, S. E., Tygstrup, N. & Lundquist, F. Metabolism of fructose in the isolated perfused pig liver. Acta med Scand. Suppl. 000 000, 1972.
13. Stuhlfaut, K. & Neumaier H. Die Wirkung der Laevulose auf Alkoholintoxikationen. Med. Klin. 46 591 1951
14. Thieden, H. I. D. & Lundquist, F. The influence of fructose and its metabolites on ethanol metabolism in vitro. Biochem. J 102. 177 1967
15. Thieden, H. I. D., Grunnet, N. Damgaard, S. E. & Sestoft, L. Effect of fructose and glyceraldehyde on ethanol metabolism in human and rat liver. Eur. J. Biochem. (In print)
16. Tygstrup, N. Winkler K. & Lundquist, F. The mechanism of the fructose effect on the ethanol metabolism of the human liver. J. clin. Invest. 44 817 1965
17. Westerfeld, W. W. Stotz, E. & Berg, R. L. The rol of pyruvate in the metabolism of ethyl alcohol. J. biol. Chem. 144 657 1942
18. Williamson, J. R., Scholz, R., Browning, E. T. Thurman, R. G. & Fukiami, Miriam H. Metabolic effects of ethanol in perfused rat liver. J. biol. Chem. 244 5044, 1969

Acta med. scand. Suppl. 54.., pp. 141—150

MODIFICATION OF METABOLIC EFFECTS OF ETHANOL BY FRUCTOSE

Reino H. Ylikahri, Matti T Kähönen and Ilmo Hassinen

From the Department of Medical Chemistry University f Helsinki, Helsinki, Finland

Abstract. Present knowledge of the metaboli interactions between fructose and ethanol in man and laboratory animals is reviewed. The possibl biochemical mechanisms of these interactions are considered. Some new data on the effects of fructose and ethanol on carbohydrate and lipid metabolism are included.

Fructose increases the effects of ethanol on the hepatic lactate/pyruvate and α-glycerophosphate/dihydroxyacetone phosphate ratios. Thus it augments the basic metabolic effect f ethanol, i.e. reduction of the hepatic redox state.

The ethanol-induced increase in hepatic α-glycerophosphate concentration has been regarded as an important factor in the pathogenesis f the acute alcoholic fatty liver But although fructose causes hypertriglyceridaemia and augments the effect of ethanol on hepatic α-glycerophosphate concentration, it was found to diminish the ethanol induced accumulation of triglycerides in the liver Glucose had similar effect. The reason for this is uncertain, but the inhibition of peripheral lipolysis by fructose and glucose might be partly responsible

The most important effect f ethanol on carbohydrate metabolism is to inhibit hepatic gluconeogenesis by producing NADH in excess. Although the production of glucose from fructose is not directly dependent on the redox state f the liver it is slightly inhibited by ethanol in vitro However in vivo fructose is effectively converted t glucose even during ethanol oxidation, and in ethanol induced hypoglycaemia it rapidly restores the blood glucose level

Fructose is one of the few compounds reported to accelerate the elimination of ethanol at least in some metabolic states (12, 47 51, 53 54). Much effort has been expended in endeavours to elucidate the mechanism of this effect. Because these studies throw some light on the metabolic effects of ethanol, we have a reasonable amount of data about the metabolic interactions of fructose and ethanol in man and laboratory animals. These interactions have been studied especially by Lundquist et al. (35—37 53, 54), by Papenberg et al. (46) and by Hassinen et al. (18, 20 57 58).

The aim of this paper is to survey the effects of fructose on ethanol induced changes in the hepatic redox state and on the alterations in carbohydrate and lipid metabolism occurring during ethanol oxidation.

EFFECTS OF FRUCTOSE AND ETHANOL ON THE REDOX STATE OF THE LIVER NAD/NADH COUPLE

We do not fully understand the mechanism which regulates the rate of ethanol metabolism. However the ethanol-induced accumulation of reducing equivalents in the liver has generally been regarded as an important factor at least *in vivo* (24, 50). Many workers have investigated the effect of fructose on the ethanol-induced reduction of the hepatic redox state in the hope of throwing further light on the way in which fructose increases the rate of elimination of ethanol.

Fructose itself alters the equilibrium of the different redox couples in the liver to a more reduced state, as indicated by the increase of the hepatic lactate/pyruvate (L/P), malate/oxaloacetate (MAL/OAA) and α-glycerophosphate/dihydroxyacetone phosphate (α-GP/DAP) concentration ratios (5 22, 48) Surface fluorometric measurements on the perfused rat liver also show that NADH fluorescence increases transiently after addition of fructose (19). A possible mechanism for the fructose-induced redox changes is discussed by Hassinen et al. in this same symposium (21).

Data about the effect of fructose on the ethanol-induced reduction of the hepatic NAD/NADH ratio are somewhat contradictory In 1956 Holzer and Schneider suggested that fructose might accelerate the rate of ethanol oxidation by functioning as a hydrogen acceptor (25). They had no experimental data to support this hypothesis. In experiments on liver slices, Thieden and Lundquist found that fructose really does reduce the ethanol induced increase in the hepatic L/P ratio (53) However the L/P ratios in their control experiments were abnormally high (about 50). In experiments on perfused livers Papenberg et al. (46) found that fructose significantly enhanced the ethanol induced redox changes, as indicated by the hepatic L/P and α-GP/DAP ratios. Ylikahri et al. (58) found that the L/P ratio in the perfusion medium was significantly higher when livers were perfused with fructose and ethanol than when they were perfused with glucose and ethanol (Fig 1). The results of *in vivo* experiments are qualitatively similar When fructose and ethanol were administered intravenously to anaesthetized rats the hepatic α-GP/DAP ratio was increased significantly more than with ethanol alone (45). The same fact was observed when fructose

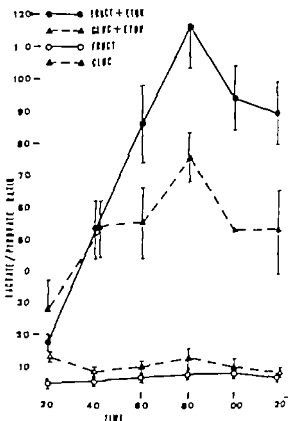


Fig 1 Lactate/pyruvate concentration ratio (L/P) of the perfusion medium after addition of different substrates. The livers of normal fasted rats were perfused with 100 ml of Krebs-Ringer bicarbonate solution t pH 7 4 temperature 35 C, for 20 minutes without any substrate 1 mmole of fructose or glucose with or without 2 mmoles of ethanol was then added. Each point represents the mean ± SEM of the values from 6 to 8 perfusions of different livers.

Table I. *Production or elimination of metabolites in liver perfusion during the first hour after addition of substrates*

Livers were perfused with Krebs-Ringer bicarbonate solution as described earlier (56). After 20 minutes of perfusion 10 mM glucose or 10 mM fructose with or without 20 mM ethanol was added to the perfusion medium. Results are expressed as μmoles/100 g body wt/hour. Values are means ± SEM. Number of experiments in parenthesis

Substrate added	Metabolite produced (+) eliminated (—): Glucose	Fructose	Lactate	Pyruvate
Glucose	— 60.8 ± 13.9 (5)	—	+ 14.0 ± 1.4 (5)	+ 1.8 ± 0.9 (5)
Glucose + ethanol	— 60.8 ± 9.7 (5)	—	+ 29.4 ± 7.1 (5)	+ 0.5 ± 0.1 (5)
Fructose	+ 109 ± 19 (7)	— 291 ± 10 (5)	+ 101 ± 8 (5)	+ 13.9 ± 1.8 (5)
Fructose + ethanol	+ 124 ± 9 (12)	— 258 ± 34 (6)	+ 118 ± 12 (7)	+ 1.1 ± 0.2 (7)

(or glucose) was given to rats by stomach tube at the same time as ethanol (Fig. 5). Thus fructose seems to augment the ethanol induced reduction of the redox state of the hepatic NAD/NADH couple.

The reduction of the hepatic NAD/NADH ratio is the most important metabolic effect of ethanol (13, 28, 32). Almost all the metabolic disturbances produced by ethanol have been linked to this redox change (13, 28, 32). Now it appears possible that when fructose affects this basic change, it also alters other metabolic effects of ethanol, for instance ethanol induced changes in the metabolism of carbohydrates and lipids. These points will be discussed in the latter part of this paper.

FRUCTOSE AND ETHANOL-INDUCED CHANGES IN HEPATIC CARBON DIOXIDE PRODUCTION

The normal liver rapidly converts ethanol to acetate, which is transported to other tissues and there oxidized (see ref. 13). The oxidation of ingested ethanol accounts for more than 70 per cent of the energy requirements of the liver (see ref. 13). Hepatic oxygen consumption is unchanged, which means that ethanol monopolizes hepatic energy metabolism. Hepatic CO_2 production is inhibited and the respiratory quotient decreases to very low values (15, 55). This means that ethanol inhibits the function of the citric acid cycle. The basic biochemical mechanism of this effect is unsolved, but one important factor is probably the ethanol-induced accumulation of NADH in the liver (13, 55). In support of this hypothesis Lindros (33) found a negative correlation between the L/P ratio and the respiratory quotient in perfused liver.

Fructose is also rapidly metabolized in the liver (Table I) (49, 58) and affects the ethanol induced changes in the hepatic redox state, but we could find no reliable information on how fructose affects the ethanol induced changes in liver CO_2 production. Fructose alone has been reported to increase CO_2 production of the liver (54). In human experiments ethanol with fructose depressed hepatic CO_2 production even more than ethanol alone (54). Forsander and Himberg found that in high concentrations glucose inhibited the ethanol-induced decrease in the hepatic respiratory quotient (14), but they did not test fructose.

FRUCTOSE AND ETHANOL-INDUCED CHANGES IN CARBOHYDRATE METABOLISM

Ethanol is known to affect the blood glucose level (4, 38, 52). The direction of the

change depends on the metabolic state of the subject (see references 13 and 38). These effects are also considered by Hillbom et al. in this Symposium (23). In fasted subjects ethanol causes hypoglycaemia owing to inhibition of hepatic gluconeogenesis by excess NADH (11 16 38). In fed subjects ethanol increases the blood glucose concentration (52), owing to ethanol induced glycogenolysis mediated by a neurohumoral mechanism (see ref. 38).

In the normal liver fructose is rapidly phosphorylated to fructose-1-phosphate and metabolized via glycolysis much faster than glucose, as indicated by increased lactate production (Table I) (49 58). A considerable part of fructose is converted to glucose. Gluconeogenesis from fructose, unlike that from pyruvate and alanine, is not directly dependent on the redox state of the liver because there is no dehydrogenation step in this pathway (29).

Reports on the combined effects of fructose and ethanol on liver carbohydrate metabolism are contradictory. Madison et al. (39) found that ethanol did not inhibit the conversion of fructose to glucose and that fructose effectively raised the blood glucose level during alcohol-induced hypoglycaemia, although glutamate and α-ketoglutarate had no effect. In perfused livers Exton and Park found that gluconeogenesis from fructose was not significantly inhibited by ethanol (10). Krebs et al. used perfused livers, too, but found that ethanol markedly inhibited production of glucose from fructose (29). Our results confirm this (Table I) (58). Papenberg et al. (46) also found that in perfused rat liver ethanol inhibited uptake of fructose and output of glucose. The inhibition found by several authors may be due to the fact that during ethanol oxidation the DAP formed from fructose-1 phosphate in a reaction catalyzed by aldolase B is rapidly converted to its reduced form, α-GP which is used for triglyceride synthesis (57).

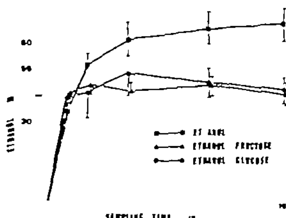


Fig. 2. Blood ethanol concentrations after administration of ethanol with glucose or fructose. Ethanol (5.0 g/kg body wt) alone or with glucose (8.7 g/kg body wt) or fructose (8.7 g/kg body wt) was administered to normal fed rats by stomach tube. Blood samples were taken from the tip of the tail. Each point represents mean ± SEM of the values from 8 rats.

In the intact organism the interactions of fructose and ethanol are even more complex than in the isolated liver. Tygstrup et al. have performed elegant human experiments to elucidate this question (54). They found that the splanchnic output of glucose was three times as great during ethanol fructose infusion as during fructose infusion alone. Thus ethanol seemed to increase the conversion of fructose to glucose in the human organism.

We have recently made some experiments on fed rats to study the interactions of fructose and ethanol in intact animals. The rats were divided into five groups. Group I received glucose 8.7 g/kg body wt (isocaloric to 5.0 g of ethanol) by stomach tube. Group II received the same amount of fructose and group III ethanol 5.0 g/kg body wt. Groups IV and V received glucose 8.7 g + ethanol 5.0 g/kg body wt and fructose 8.7 g + ethanol 5.0 g/kg body wt. Blood samples for ethanol, fructose and glucose determinations were taken from the tip of the tail. Determinations were carried out by the methods which we have used earlier (57 58).

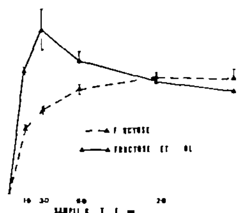


Fig. 3. Blood fructose concentrations after d ministration of fructose with or without ethanol. Experimental conditions as in Fig. 2. Each point represents mean ± SEM of the values from 8 rats.

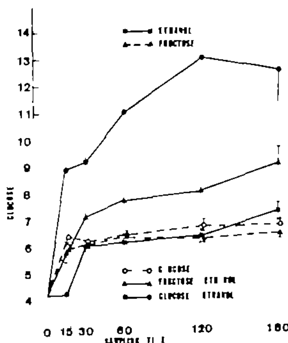


Fig. 4. Effect f oral administration f fructose, glucose and ethanol on blood glucose concentration in rats. Experimental conditions as in Fig 2. Each point represents mean ± SEM of the values from 8 rats.

Blood ethanol concentrations were significantly higher in group III than in groups IV and V (Fig. 2). But the values 15 minutes after administration were still similar in all groups. This suggests that the differences found during the latter part of the experiment are not attributable to delayed absorption of ethanol in groups IV and V but to the acceleration of ethanol elimination by fructose and glucose.

Fructose concentrations in peripheral blood were low all the time (Fig 3). This is probably due to the rather slow absorption of fructose and its rapid uptake by the liver. During the first hour after administration the concentrations were higher in the fructose + ethanol group than in the fructose group. We cannot as yet offer any explanation for this phenomenon.

Blood glucose concentrations were highest in group IV (glucose + ethanol) (Fig 4). This indicates that glucose combined with ethanol increases blood glucose much more than glucose alone. Ethanol hardly affects glucose absorption, but it causes glycogenolysis in the liver and thus raises the blood glucose level. Ethanol has also been reported to inhibit peripheral utilization of glucose (34). This may account for the high blood glucose concentrations in all groups of experimental animals which had received ethanol.

Fructose alone increased the blood glucose concentration (Fig 4). Ethanol augmented this effect, indicating that fructose is rapidly converted to glucose even during ethanol metabolism.

It can be concluded that ethanol may slightly inhibit the production of glucose from fructose *in vitro* but that *in vivo* fructose rapidly abolishes the hypoglycaemia induced by ethanol.

EFFECT OF FRUCTOSE ON ACUTE ETHANOL-INDUCED FATTY LIVER

The mechanism by which one large dose of ethanol induces the accumulation of tri

glycerides in the liver has been studied intensively Some investigators attribute the phenomenon to the effect of ethanol on peripheral lipolysis or to direct toxic effects of ethanol on the liver or to both these mechanisms (2, 6 8 9 26). Others hold that ethanol per se does not produce fatty liver but that the changed metabolic con ditions in the liver during the oxidation of ethanol favour triglyceride synthesis (27 41 42, 56). The increased formation of triglycerides is assumed to be linked with the increased liver NADH content. The reduc tion of NAD to NADH favours the reduc tion of DAP to α-GP which has been con sidered as an important regulator of hepatic lipogenesis (17 40—42, 56) Studies on rats treated with pyrazole, which effectively in hibits the oxidation of ethanol, have indi cated that ethanol oxidation is necessary for the induction of acute alcoholic fatty liver (1, 7 40). Results from thyroxine-treated and clofibrate-treated rats also suggest that ethanol-induced reduction of the hepatic redox state is essential in the pathogenesis of acute alcoholic fatty liver (3 30 31 56). Thyroxine and clofibrate prevent the ethanol-induced redox change, and they also inhibit the production of fatty liver by ethanol (30 31 56).

Several reports show that fructose, even alone but especially with ethanol, increases the hepatic content of α-GP and so stimu lates triglyceride synthesis (5, 41, 42, 56, 59). Moreover fructose has been found to induce triglyceridaemia in man and laboratory animals (43 44). Thus it might be expected that fructose would worsen the alcoholic fatty liver

To clarify this question we studied the effect of fructose on ethanol induced hepatic triglyceride accumulation. Five groups of rats were treated as described on page 000 (I glucose, II fructose, III ethanol, IV glucose + ethanol, V fructose + ethanol) The ani mals were anaesthetized 4 or 12 hours after

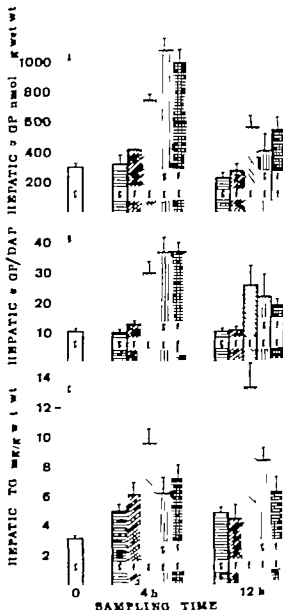


Fig. 5 Effects of fructose, glucose and ethanol on hepatic α-glycerophosphate (α-GP) concentration, hepatic α-glycerophosphate/dihydroxyacetone phosphate (α-GP/DAP) ratio and hepatic triglyceride (TG) concentration in rats. Rats were treated as in Fig. 2. Liver samples were taken under light ether anaesthesia and placed in liquid nitrogen. Symbols: C = control (no treatment), G = treated with glucose, F = treated with fructose, E = treated with ethanol, G + E = treated with glucose + ethanol, F + E = treated with fructose + ethanol. Each bar represents mean ± SEM of the values from 6 to 8 rats.

the treatment, the abdomen was opened and a piece of liver was removed to liquid nitrogen for α-GP DAP and triglyceride determinations. Immediately afterwards the abdominal aorta was cut and blood collected for determination of serum triglycerides and free fatty acids. The methods used have been described earlier (57 58)

As seen in Fig 5A, ethanol caused a significant rise in hepatic α-GP concentration at 4 hours. Both fructose and glucose augmented this effect significantly Twelve hours after the treatment α-GP concentrations were still increased in the ethanol groups, but no additive effect of fructose was observed.

The curves for the α-GP/DAP ratios were parallel to those of α-GP (Fig 5B) because the DAP concentration was almost unchanged during the experiment. Thus during acute intoxication both fructose and glucose augmented the redox effect of ethanol.

Both fructose and glucose increased the hepatic triglyceride content (Fig 5C) but the effect of ethanol was significantly greater It was somewhat surprising to find that in both the glucose + ethanol and fructose + ethanol groups the hepatic triglycerides rose far less than in the ethanol group, although the α-GP concentration was higher in the two first-mentioned groups. In all groups which had received ethanol the changes in serum triglyceride concentrations were about the same (Fig 6A). Serum free fatty acid concentration was increased by ethanol but decreased by ethanol + glucose and ethanol + fructose (Fig. 6B). This decrease is probably attributable to inhibition of peripheral lipolysis by high serum glucose concentrations.

The present results show that although the reduction of the hepatic redox state is one of the essential changes underlying the production of fatty liver by ethanol, it is not the only important factor These experiments do not throw light on the mechanism by which fructose and glucose inhibit the production of fatty liver by ethanol. Inhibition of peripheral lipolysis is certainly one facto but probably not the only one. Domanski et al. have also found that glucose is protective against the production of fatty liver by ethanol, but they offer no explanation for this effect (7).

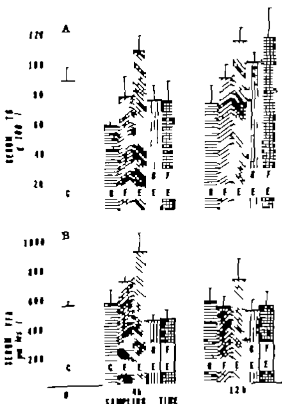


Fig 6 Effects of glucose fructose and ethanol on serum triglyceride (TG) and free fatty acid (FFA) concentrations in fed rats. Rats were treated as in Fig. 2. Blood samples were taken under light ether anaesthesia by cutting the abdominal orta Each bar represents mean ± SEM of the values from 6 to 8 rats.

The doses of fructose and glucose used in these experiments were large. A more detailed study including dose-response relationships, is needed to solve the problem of how fructose and glucose prevent ethanol-induced fatty liver

REFERENCES

1. Blomstrand, R. & Forsell, L. Prevention of the acute ethanol-induced fatty liver by 4-methyl pyrazole. Life Sci. 10. II 523, 1971.
2. Bode, Ch., Stähler E., Kono, H. & Goebell, H. Effects of ethanol on free coenzyme A, free carnitine and their fatty acid esters in rat liver Biochim. biophys. Acta 210 448, 1970
3. Brown, D. F The effect of ethyl-α p-chlorophenoxyisobutyrate on ethanol-induced hepatic steatosis in the rat. Metabolism 15 868, 1966.
4. Brown, T McP & Harvey A. M., Spontaneous hypoglycemia in »smoke» drinkers. J.A.M.A. 117 12, 1941.
5. Burch, H. B Lowry O H., Meinhardt, L., Max, P Jr & Chyu, K. Effect of fructose dihydroxyacetone, glycerol and glucose on metabolites and related compounds in liver and kidney J biol. Chem. 245 2092, 1970
6. Bustos, G O Kalant, H., Khanna, J M. & Loth, J Pyrazole and induction of fatty liver by a single dose of ethanol. Science 168 1598, 1970.
7. Domanski, R., Rifenberick, D Stearns, F Scorpio, R. M. & Narrod, S. A. Ethanol induced fatty liver in rats Effects of pyrazole and glucose Proc Soc. exp. Biol. Med. 138 18, 1971
8. Estler C. J & Ammon, H. P T The influence of beta-adrenergic blockade on the ethanol induced derangement of lipid transport. Arch. int. Pharmacodyn. 166 333 1967
9. Estler C.-J & Ammon, H P T The influence of antilipolyti gents (KO 592 and β-pyridylcarbinol) on lcohol induced deposi tion of fat in the li er p 207 in Martin, G A. and Bode Ch. (Eds) Metabolic Changes Induced by Alcohol. Springer Verlag, Berlin, 1971.
10. Exton, J H. & Park, C R Control of gluconeogenesis in liver II Effect of glucagon, catecholamines, nd adenine 3,5-monophosphate on gluconeogenesis in the perfused rat liver J biol. Chem 243 4189 1969
11. Field, J B Williams, H E & Mortimore, G E. Studies on the mechanism of ethanol induced hypoglycemia J clin. Invest. 42 497 1963.
12. Forney R. B. & Harger R. N Toxicology of ethanol. Ann. Rev Pharmacol. 9 397 1969
13. Forsander O A. Effects of ethanol on metabolic pathways. In Tremolieres, J (Ed) International Encyclopedia of Pharmacology and Therapeutics. Sect. 20 Vol. 1 p. 117. Pergamon Press, New York, 1970
14. Forsander O A. & Himberg, J J Effect of glucose on liver metabolism during ethanol oxidation. Metabolism 18 776, 1969.
15. Forsander O A., Räihä, N., Salaspuro, M. & Mäenpää, P Influence of ethanol on the liver metabolism of fed and starved rats Biochem. J 94 259 1965.
16. Freinkel, N Singer D L., Arky R. A Bleicher S. J Anderson, J B & Silbert, C. K. Alcohol hypoglycemia. I. Carbohydrate metabolism of patients with clinical alcohol hypoglycemia and the experimental reproduction of the syndrome with pure ethanol. J clin. Invest. 42 1112, 1963.
17. Fritz, I. B. Factors influencing the rates of long chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev 41 52, 1961
18. Hassinen, I. Metabolic interactions of fructose, glycerol and ethanol. Ann. Med. exp Biol. Fenn. 42 76, 1964.
19. Hassinen, I. & Ylikahri, R. H. Absorption spectrophotometry of perfused rat liver applied to fructose-induced inhibition of respiration. Biochem. biophys. Res. Commun. 38 1091 1970
20. Hassinen, I. E., Ylikahri, R. H. & Kähönen, M. T.. Effect of ethanol, thyroxine and fructose on the intracellular redox state of a perfused liver studied by surface fluorometry Ann. Med. exp. Fenn. 48 176, 1970.
21. Hassinen, I Ylikahri, R. H. & Kähönen, M. T Effect f fructose on cellular respiration in perfused rat liver Acta med. scand. this supplement, pp. 105—110
22. Heinz, F & Junghänel, J Metabolitmuster in Rattenleber nach Fructoseapplikation. Hoppe-Seyler's Z. physiol. Chem. 350 859, 1969
23. Hillbom, M., Lindros, K. O & Eriksson, P Effects of fructose, glucose and glyceraldehyde on the toxicity f ethanol in hyperthyroid rats. Acta med. scand. this supplement, pp. 151—155.
24. Hillbom, M. E. & Pikkarainen, P H. Liver alcohol and sorbitol dehydrogenase activities in hypo- and hyperthyroid rats. Biochem. Pharmacol. 19 1006, 1970
25. Holzer H. & Schneider S. Zum Mechanismus der Beeinflussung der Alkoholoxydation in der Leber durch Fructose. Klin. Wschr 33 1006 1955.
26. Horning, M. G Williams, E. A Maling, H. M. & Brodie, B. B. Depot fat as source of

increased liver triglycerides after ethanol. Biochem. biophys. Res. Commun. 3 635 1960.

27 Isselbacher K. J & Greenberg, N J Metabolic effects of alcohol on the liver New Engl. J Med. 270 402, 1964.

28. Krebs, H. A. The effects of ethanol on the metabolic activities of the liver In Weber G (Ed) Advances in Enzyme Regulation. Vol 6. p. 467 Pergamon Press, New York, 1968.

29. Krebs, H. A., Freedland, R. A., Hems, R. & Stubbs, M.. Inhibition of hepatic gluconeogenesis by ethanol. Biochem. J 112 117 1969

30 Kähönen, M. T Ylikahri, R. H. & Hassinen, L. Ethanol metabolism in rats treated with ethyl-a-p-chlorophenoxyisobutyrate (clofibrate) Life Sci. 10 II 661 1971.

31. Kähönen, M. T Ylikahri, R. H. & Hassinen, L. Studies on the mechanism of inhibition of acute alcoholic fatty liver by clofibrate. Metabolism (in press).

32 Lieber C. S.. Alcohol and the liver In Bittar E. E. (Ed.) The Biological Basis of Medicine. Vol. 5 p. 317 Academic Press, London, 1969

33 Lindros, K. O Supression by ethanol of acetate and hexanoate oxidation studied by nonrecirculating liver perfusion. Biochem. biophys. Res. Commun. 41 635, 1970.

34. Lochner A., Wulff, J & Madison, L. L. Ethanol induced hypoglycemia. I. The acute effects of ethanol on hepatic glucose output and peripheral utilization in fasted dogs. Metabolism 16 1, 1967

35. Lundquist, F Metabolism of carbohydrates in the normal liver under the influence of ethanol and hormones. Scand. J clin Lab. Invest. 18, suppl. 92 47 1966.

36. Lundquist, F Svendsen, L & Petersen, P H The metabolism of ethanol in rat liver suspensions. Biochem. J 86 119, 1963

37 Lundquist, F & Wolthers, H. The influence f fructose on the kinetics of alcohol elimination in man. Acta pharmacol. Toxicol. 14 290, 1958.

38. Madison, L. L. Ethanol-induced hypoglycemia. In Levine, R. and Luft, R. (Eds.) Advances in Metabolic Disorders. Vol. 3. p. 85. Academic Press, New York, 1968.

39 Madison, L. L., Lochner A. & Wulff, J Ethanol-induced hypoglycemia II. Mechanism of suppression of hepatic gluconeogenesis Diabetes 16 252, 1967

40. Morgan, J C. & DiLuzio, N R. Inhibition of the acute ethanol induced fatty liver by pyrazole Proc. Soc. exp. Biol. Med. 134 462, 1970.

41. Nikkilä, E. A. & Ojala, K. Role of hepatic L-α-glycerophosphate and triglyceride synthesis in production of fatty liver by ethanol. Proc. Soc. exp Biol. Med. 113 814, 1963.

42 Nikkila, E A. & Ojala, K. Ethanol induced alterations in the synthesis of hepatic and plasma lipids and hepatic glycogen from glycerol-C[14] Lif Sci. 2 717 1963

43. Nikkila, E A & Ojala, K Induction of hyperglyceridemia by fructose in the rat. Life Sci 4 937 1965

44 Nikkila, E A & Pelkonen, R. Enhancement of alimentary hyperglyceridemia by fructose and glycerol in man. Proc Soc. exp Biol. Med 123 91 1966

45. Nikkila, E A & Ylikahri, R H Unpublished results

46 Papenberg, J von Wartburg, J P & Aebi, H Metabolism of ethanol and fructose in the perfused rat liver Enzym. Biol. Clin. 11 237 1970

4 Pawan, G L. S Vitamins, sugars and ethanol metabolism in man Nature (London) 220 374 1968

48 Raivio, K O Kekomaki, M. P & Maenpaa, P H. Depletion f liver adenine nucleotides induced by fructose Dose-dependence and specificity of the fructose effect. Biochem. Pharmacol 18 2615 1969

49 Renold, A. E Hastings, A. B. & Nesbett, F B Studies on carbohydrate metabolism in rat liver slices. III. Utilization f glucose and fructose by liver from normal and diabetic animals. J biol. Chem. 209 687 1954.

50 Smith, M E & Newman, H. W The rate of ethanol metabolism in fed and fasting animals. J biol. Chem. 234 1544, 1959

51 Stuhlfauth, K. & Neumeyer H. Di Wirkung der Laevulose auf Alkoholintoxikationen. Med. Klin. 46 591, 1951.

52. Tennet, D M. Factors influencing the effects of alcohol on blood sugar and liver glycogen. Quart. J Stud. Alc. 2 263, 1941.

53. Thieden, H. I. D & Lundquist, F The influence f fructose and its metabolites on ethanol metabolism in vitro. Biochem. J 102 177 1967

54 Tygstrup, N Winkler K. & Lundquist, F.. The mechanism of the fructose effect on the ethanol metabolism of the human liver J clin. Invest. 44 817 1965

55. Williamson, J R., Scholz, R., Browning, E. T Thurman, R. G & Fukami, M. H.. Metabolic effects of ethanol in perfused rat liver J biol. Chem. 244 5044, 1969

56. Ylikahri, R. H. Ethanol induced changes in hepatic α-glycerophosphate and triglyceride concentrations in normal and thyroxine-treated rats. Metabolism 19 1036, 1970
57. Ylikahri, R. Metabolic effects of ethanol and fructose in thyroxine-treated rats. Helsinki 1970 (Thesis)
58. Ylikahri, R. H., Hassinen, I. & Kähönen, M. T. Metabolic interactions of fructose and ethanol in perfused liver of normal and thyroxine-treated rats. Metabolism 20 555, 1971
59. Zakim, D. & Herman, R. The effect of intravenous fructose and glucose on the hepatic α-glycerophosphate concentration in the rat. Biochim. biophys. Acta 165 374, 1968.

Acta med. scand. Suppl. 54.., pp. 151—155

EFFECTS OF FRUCTOSE, GLUCOSE AND GLYCERALDEHYDE ON THE TOXICITY OF ETHANOL IN HYPERTHYROID RATS

M. E. Hillbom, K. O Lindros and C J P Eriksson

From Research Laboratories of the State Alcohol Monopoly (Alko), 00101 Helsinki 10 F land

Abstract. The effects of singl or repeated intraperitoneal injections of fructose and glyceraldehyde were compared with those of glucose and saline in the treatment and prevention of the toxicity of ethanol in hyperthyroid rats. Glyceraldehyde potentiated the lethal effect of ethanol on these animals. The dose of ethanol (3 g/kg body wt.) produced only slight ketosis. Fructose, despite of its antiketogenic action, was equal in value to glucose or saline in the treatment of the intoxication syndrome. Ethanol induced hypoglycemia, which was previously assumed to be causally related to the toxicity of ethanol in hyperthyroid rats, was counteracted by giving repeated intraperitoneal injections of fructose or glucose, but the mortality f the animals was the same as in saline-treated controls. The results suggest that hypoglycemia is not the principal cause of death in ethanol-intoxicated hyper thyroid rats.

It has been shown that ethanol may especial ly after fasting (5) or in the hyperthyroid state (1), induce severe hypoglycemia which sometimes leads to coma or even death. A rapid reversal of this condition can be achieved with injections of glucose, at least in the case of ethanol-induced hypoglycemia of the type developing after fasting and poor nutrition (7 16) This treatment has not been tried in hyperthyroid patients devel oping hypoglycemia after ethanol. In ex periments with hyperthyroid rats Ylikahri (20) has shown that large doses of ethanol produce severe hypoglycemia soon after administration and we have previously demonstrated that ethanol also induces slight ketosis in these animals (11) although gener ally described as an antiketogenic agent. The role of these effects in the toxicity of ethanol in the hyperthyroid state is not established.

In order to test the possibility of a causal relationship between the ethanol-induced hy poglycemia or ketosis and the toxicity of ethanol we studied the effects of single and repeated intraperitoneal injections of fructose, glucose and glyceraldehyde on ethanol induced mortality in hyperthyroid rats. Fructose was compared with glucose because of its glucogenic and antiketogenic properties and because it has been reported to stimulate the rate of ethanol oxidation in liver tissue (2, 17). In liver perfusion experiments it has also been demonstrated

that gluconeogenesis from fructose is not inhibited by ethanol in the hyperthyroid state (21)

MATERIAL AND METHODS

Fed male Wistar rats, four months of age and weighing from 250 to 250 g were used in the experiments. All the animals were given an ordinary laboratory diet (containing 34 / of calories as protein) and tap water ad libitum. The animals were rendered hyperthyroid by injecting 0.2 mg/kg body weight of 3,3',5-triiodo-L-thyronine (T_3) in slightly alkaline solution intraperitoneally on seven successive days. The experiments were performed on the eighth day

In the first part of the study thirty two rats were given 3 g/kg body weight of ethanol intraperitoneally in saline (20 / w/v solution) Four of them died within half an hour and the remaining twenty-eight animals were randomly divided into four groups of seven rats and immediately given glyceraldehyde (1 g/kg body wt.) fructose (1 g/kg body wt.), glucose (1 g/kg body wt.) or saline (equal volume). Levels of blood glucose and ketone bodies (β-OH butyrate + acetoacetate + acetone) were determined before giving ethanol and one half, one and two hours after its administration. The injections of glyceraldehyde, fructose, glucose and saline were given intraperitoneally just fter the blood sample taken half an hou after the administration of ethanol. The time course of mortality of the animals was recorded and values of blood glucose and total ketone bodies were measured by methods described elsewhere (4 19).

In a second series of eighteen rats, divided into three groups we tudied the effects of repeated injections of fructose, glucose or saline on the mortality of hyperthyroid rats. The first group of animals was given a single intraperitoneal injection of a 20 / (w/v) ethanol (3 g/kg body wt.) solution in saline. The other two groups received the same amount of ethanol together with fructose or glucose (1 g/kg body wt.). Thereafter five intraperitoneal injections of fructose (1 g/kg body wt.), glucose (1 g/kg body wt) or saline (equal volume) were given hourly Blood glucose and ethanol values were determined on the surviving rats six hours after the injection of ethanol. Blood samples were taken from the tip of the tail and blood alcohol was determined by gas chromatograph as described earlier (8).

RESULTS AND DISCUSSION

The results indicate (Table I) that the dose of ethanol used in these experiments was lethal when given intraperitoneally to hyper thyroid rats, thus confirming the previous report of Ylikahri (20) In euthyroid rats the LD_{50} for intraperitoneal injection of a 10 or 20 % (w/v) solution in saline is given as 5 g/kg (18)

A single intraperitoneal injection of 5 • glyceraldehyde solution was found to potentiate the toxicity of ethanol in hyperthyroid rats. All the animals in this group died within one and a half hours after the in jection but most of the animals given glucose fructose or saline survived for more than three hours. When the same dose of glyceraldehyde was given to four hyper thyroid animals without ethanol none of them died. The last blood samples taken

Table I *Effect of a single intraperitoneal injection of glyceraldehyde fructose glucose or saline on the ethanol induced mortality of hyperthyroid rats*

Treatment	Number of animals	Died within /₂—3 hr	Died within 3—6 hr	Survived
Glyceraldehyde (1 g/kg body wt)	7	7	—	—
Fructose (—-—)	7	2	5	—
Glucose (—-—)	7	2	5	—
Saline (equal volume)	7	2	5	—

Thirty-two fed male Wistar rats were given ethanol (3 g/kg body wt.) intraperitoneally in saline and four of them died within half an hour The remaining twenty-eight animals were randomly divided into four groups of seven rats and immediately given glyceraldehyd fructose, glucose or saline

Table II. *Blood glucose levels in hyperthyroid rats after injection of 3 g/kg ethanol intraperitoneally*

Group	Number of animals	Blood glucose	
		Before ethanol injection	Last value before death
Surviving more than 3 hr	15	7.1 ± 0.6	7.4 ± 1.8
Surviving less than 3 hr	13	6.8 ± 0.6	4.7 ± 1.1

The animals are the same as those described in Table I. The figures represent the mean ± S.D. Blood glucose values are expressed as nmoles/ml of blood.

Table III. *Blood ketone body levels in hyperthyroid rats after injection of 3 g/kg ethanol intraperitoneally*

Group	Number of animals	Blood ketone bodies	
		Before ethanol injection	Last value before death
Surviving more than 3 hr	15	164 ± 47	231 ± 66
Surviving less than 3 hr	13	167 ± 65	275 ± 86

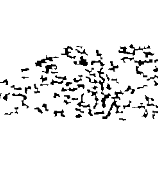
The animals are the same as those described in Table I. The figures represent the mean ± S.D. Values of total ketone bodies (β-OH-butyrate + acetoacetate + acetone) are expressed as nmoles/ml of blood.

from the glyceraldehyde-treated animals given ethanol revealed slight hypoglycemia in every case, but similar blood glucose values were also obtained from animals treated with fructose, glucose or saline, half an hour before death ensued. The values recorded from the total material treated with single injections indicated that the fatal outcome after ethanol was in fact preceded by a significantly ($p < 0.001$) lowered level of blood glucose as can be seen in Table II. However it must be pointed out that in most animals the lowered blood glucose level prevailed for only a short period before death. A single injection of fructose or glucose did not prolong the life of the animals as compared with the saline-treated controls, although a transient rise in blood glucose was noted after their administration.

An examination of the development of blood ketone bodies in those animals which survived for more than three hours and those which survived for less than three hours indicated an equal and significant ($p < 0.01$) increase in both groups as can be seen in Table III. However this increase was rather slight and the level of ketone bodies was not influenced to any great extent by the single injections of fructose, glucose or glyceraldehyde. In any case it can be concluded that the fatal outcome after ethanol is not connected with severe ketosis in the hyperthyroid state.

Heim and his coworkers have reported that the lethal dose of ethanol is doubled in euthyroid cats when 4 g/kg body wt. of fructose is given before the experiment (8, 9). The mechanism of this effect is unresolved. It is generally accepted that fructose can accelerate the rate of elimination of ethanol in the body (3, 12, 14, 16). However fructose has no marked effect unless it is present in the blood throughout the degradation of ethanol, and therefore must be used in large amounts.

In order to see if a large amount of



FRUCTOSE AND DIABETES

Acta med. scand Suppl. 542, pp. 159—164

EFFECT OF FRUCTOSE AND OTHER SUGARS ON ISLET FUNCTION IN VITRO

Diana Hager Daniel G Pipeleers, Andre Herchuelz and Willy J Malaisse

From the Laboratory of Experimental Medicine Brussels University Brussels Belgium

Abstract. Glucose and mannose stimulate insulin release, insulin biosynthesis and calcium uptake in rat isolated islets. Their insulinotropic action is enhanced by caffeine or theophylline. Ribose and xylitol might also exert a minor insulinotropic action. Fructose *and galactose do not stimulate* insulin release whether in the presence or absence of methylxanthine (10 0 mM) and do not affect insulin biosynthesis or calcium uptake by the islets. These converging findings on several parameters of islet function support the concept that the insulinotropic effect of sugars is dependent on their intracellular metabolism, or possibly on the activation of highly-selective glucoreceptor

The effect of fructose on insulin release has been the subject of a number of investigations. Except in one study (42), little or no direct insulinotropic action of fructose was found in vitro with either pieces of rabbit pancreas (4), pieces of rat pancreas (28) rat isolated islets (19) and the perfused rat pancreas (13, 41) Also in vivo small to insignificant changes in the level of circulating insulin were seen after oral or intravenous administration of fructose in man (1, 9 31) dog (18) and rabbit (35) The direct insulinotropic action of fructose might be more marked in fetal pancreas and during childhood. Thus, in the preparation of cultured fetal rat pancreas, a significant insulinotropic action of fructose was noticed in the presence of caffeine (20). A transient stimulation of insulin release by fructose was seen in newborn pigs (43) and sheep fetuses (5), *although not in newborn calves* (6). In normal children or young patients with Type 1 glycogenosis, fructose injection might also provoke an early secretory response (17 33). This insulinotropic action of fructose is absent in fructose intolerance (3, 7 23). Taken as a whole, these findings suggest that (i) the direct insulinotropic action of fructose is negligible in adults and possibly more marked during childhood and (ii) the modest insulinotropic effect of fructose is dependent on the accumulation of a glycolytic metabolite below the fructose-1 phosphate-aldolase level. In more general terms, the above-mentioned findings are com-

patible with the concept that the insulinotropic action of sugars is secondary to their metabolism in the beta cell, a process conceivably influenced by developmental and/or dietary factors, as well as by hereditary metabolic defects.

This concept has been recently challenged because (i) insulin release and changes in the level of intermediates and cofactors in the beta cell might occur independently and (ii) sugars which are not metabolized (e.g galactose) or which are even potential inhibitors of glucose metabolism (e.g. glucosamine) were found to stimulate insulin release under certain experimental conditions (21 32) In the light of these recent observations, we have reexamined the influence of various sugars, including fructose, upon insulin secretion and other parameters of islet function in vitro

MATERIAL AND METHODS

All experiments were performed with either pieces of pancreas or isolated islets removed from fed albino rats, and incubated for 90 min at 37 C in bicarbonate buffered media containing albumin (5 0 mg ml) The methods used for the measurement of insulin release from either pieces (28) or islets (27 30), and insulin biosynthesis (27) and calcium uptake (30) by isolated islets have been described in detail elsewher

RESULTS

Effects of individual sugars on insulin release

Glucose stimulates insulin release in both pieces of pancreas (Fig 1) and isolated islets (28, 30) The relationship between the glucose concentration of the incubation medium and the rate of insulin secretion is characterized by a sigmoidal curve with a threshold value for the insulinotropic action of glucose at a glucose level close to 5 0 mM The maximal rate of secretion is achieved

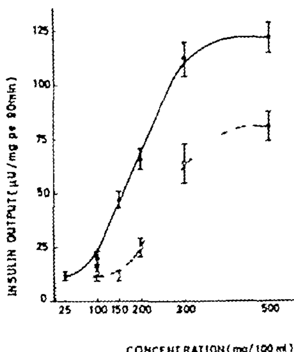


Fig 1 Mean values (± SEM) for insulin output by pieces of pancreas incubated at various glucose (closed circles solid line) or mannose (open circles dotted line) concentrations.

at concentrations ranging between 16 7 and 27.8 mM (28 30).

Mannose also stimulates insulin secretion in both pieces of pancreas (Fig. 1) and isolated islets (25 29) The effect of mannose is less marked than that of glucose at all concentrations (Fig 1)

In the present system, none of the following sugars or polyols, when used at a 16 7 mM concentration, significantly increases the basal level of insulin release galactose fructose, ribose (23), sorbitol (unpublished observations) xylitol and ribitol (23).

Combined effects of glucose and other sugars or polyols on insulin release

In the presence of a low glucose concentration (1 7 to 2.8 mM) and at high concentration (15 0 or 16 7 mM) fructose galactose

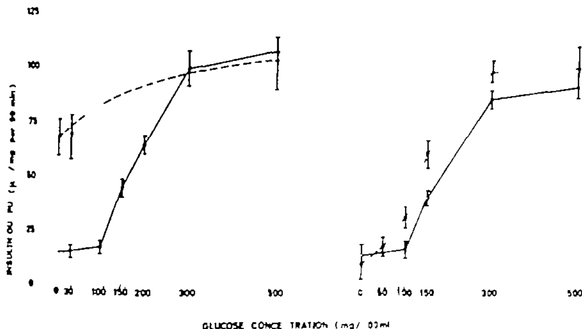


Fig. 2. The mean control values (± SEM) for insulin output by pieces of pancreas incubated at various glucose concentrations are shown as open circles (solid line). The mean changes (± SEM) in secretion rate induced by either mannose (300 mg/100 ml on the left) or xylitol ([illegible] mg/100 ml on the right) were added to substracted from their respective control values and are shown as closed circles (dotted line). Overlapping SEM refer to insignificant changes.

ribose, xylose, arabinose, 2-deoxyglucose, ribitol and xylitol also fail to significantly modify insulin release (23, 28).

At intermediate glucose concentrations (5.6 to 16.7 mM), namely in a range of glucose values where small increments in glucose concentration cause marked increases in insulin output, ribose and xylitol (16.7 mM) significantly but modestly increase glucose-induced insulin secretion (23). The effects of other sugars, under these experimental conditions, has not yet been tested in the present system.

At maximal glucose concentration (16.7 to 27.8 mM), none of the carbohydrates so far examined (i.e. mannose, ribose, xylitol and ribitol, all 16.7 mM) has any significant effect upon insulin secretion (23, 25).

As outlined in greater detail elsewhere (24) these data indicate that some carbohydrates (mannose, ribose, xylitol) mimick the effect of glucose and, by doing so, cause a shift to the left of the sigmoidal curve illustrated in Fig. 1. When the shift is of sufficient amplitude (e.g. with mannose) a significant stimulation of insulin release is observed in the absence of glucose (Fig. 2 left). Otherwise (e.g. with ribose and xylitol) a significant insulinotropic effect is only observed at intermediate glucose concentrations (Fig. 2 right).

Effects of sugars on calcium uptake and insulin biosynthesis

Glucose increases the net uptake of calcium in islets incubated in the presence of [45]calcium (30). Mannose also provokes an accumulation of [45]calcium in the islets whereas both galactose and mannoheptulose fail to significantly affect the basal calcium uptake by the isolated islets (29). These findings are consistent with the hypothesis that the insulinotropic action of various sugars is mediated through an accumulation of calcium in the beta cell (29).

Glucose, *and to a lesser extent mannose* stimulate insulin biosynthesis by isolated islets. Xylitol, fructose and galactose have no obvious effect upon insulin biosynthesis (26) These findings confirm previous observations (22)

Combined effects of sugars and methylxanthines on insulin release

Theophylline (1 4 mM) and caffeine (1.3 mM) potentiate glucose-induced insulin secretion (2, 26). In both pieces of pancreas and isolated islets, no significant effect of theophylline is seen at low glucose concentrations (0 to 2.8 mM) At higher glucose levels (5 6 to 16 7 mM), theophylline markedly enhances glucose-induced insulin release the increment in secretion rate being most marked at the highest glucose concentration (2, 26) The enhancing action of theophylline increases as its concentration in the incubation medium is raised from 0.3 to 1 4 mM (26).

Theophylline (1 4 mM) also potentiates mannose-induced insulin secretion. Again, the increment in secretion rate is much more marked at high than at low mannose concentration (25)

In more recent experiments, we have studied the effect of a higher concentration of methylxanthine (10 0 mM) upon sugar induced insulin release in isolated islets. As excepted, the methylxanthines significantly enhanced insulin release in the presence of either *glucose* or *mannose (16.7 mM)*. *In* contrast, in the absence of any sugar or in the presence of either galactose or fructose (16 7 mM) the addition of theophylline or caffeine (10 0 mM) only caused minor and usually unsignificant increases in insulin output (unpublished observations)

Methylxanthines are thought to increase insulin output by causing a translocation of calcium within the beta cell from an organelle-bound pool into the cytosol (2) In order for such translocation to cause sustained insulin release, the beta cell has to be simultaneously exposed to some other insulinotropic agent itself preventing the outward transport of the translocated calcium across the membrane of the beta cell (2). Therefore, it is not surprising that sugars which fail to provoke calcium accumulation in the beta cell also fail to support the insulinotropic action of methylxanthines.

DISCUSSION

In the present experiments, various parameters of beta cell function were influenced in parallel fashion by each of the different sugars. Thus, glucose and to a lesser extent mannose, stimulated insulin release, calcium uptake and insulin biosynthesis by the incubated islets. These sugars also allowed theophylline or caffeine to exert their insulinotropic action. In contrast, we could not detect any obvious effect of either galactose or fructose upon these various biochemical parameters. In our view these converging findings on several parameters reinforce the concept that the effect of sugars upon the beta cell function is dependent on the activation of a glucoreceptor with selective affinity for a few hexoses or more likely on the intracellular metabolism of these particular sugars.

If insulin release is due to the accumulation of a metabolic signal rather than to the activation of a poorly selective glucoreceptor *(21) it might be expected* that the pancreatic response to various sugars is influenced by constitutional and environmental factors including species and age, which might modify the enzymatic pattern of the beta cell. And, indeed, the effect of certain sugars upon insulin release appears to be affected by such factors.

We have already discussed the possible developmental influence on fructose-induced insulin release Also if there is general agreement on the failure of galactose to

directly stimulate insulin release (4 10 13 17 19 28, 31 35 37 42), the insulinotropic action of ribose and pentitols appears to be markedly influenced by a series of experimental conditions. For instance xylitol markedly stimulates insulin release in dog (15 16, 18, 44) whereas the insulinotropic action of this pentitol is small if not debatable in man (8, 29) monkey (44) and rat (20, 22, 24) Ribose might also stimulate insulin release in dog (11 14) and rabbit (35 38, 42), its effect being small and transient in man (12, 40).

In summary the present study clearly indicates that various carbohydrates, including epimers of glucose, vastly differ in their ability to stimulate insulin release and biosynthesis in the beta cell.

ACKNOWLEDGEMENTS

This study was supported in part by grants from the FRSM (Brussels, Belgium). D G P is *aspirant van de NFWO* (Brussels, Belgium)

REFERENCES

1. Aitken, J M & Dunnigan, M G Insulin and corticoid response to intravenous fructose in relation to glucose tolerance. Brit. med. J 3 276, 1969
2. Brisson, G R Malaisse-Lagae, F & Malaisse, W J The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3' 5' cyclic monophosphate. J clin Invest. 51 232, 1972.
3. Cornblath M., Rosenthal, I. M., Reisner S. H., Wybregt, S H. & Crane, R. K. Hereditary fructose intolerance. New Engl J Med. 269 1271 1963.
4. Coore, H. G & Randle, P J Regulation of insulin secretion studied with pieces of pancreas incubated in vitro Biochem. J 93 66 1964.
5. Davis, J R., Beck, P Colwill, J R., Makowski, E. L., Meschia, G & Battaglia, F C. Insulin response to fructose and gl cose infusions into the sheep fetus. Proc. Soc. exp. Biol. Med. 136 972, 1971
6. Edwards, A. V & Powers, N Effect of intravenous infusion of fructose in newborn cal es N ture (London) 214 728, 1967
7. Froesch, E R Wolf, M P Prader A., Labhart, A & Bartsch, H Hereditary fructose intolerance An inborn defect of hepatic fructose-1 phosphate splitting aldolase Amer J Med 34 151 1963
8. Gese K A F rster H Prols H & Mehnert, H Zur Frage einer Wirkung von Xylit auf di Insulinsekretion des Menschen. Klin. Wschr 45 651 1967
9. Ginsburg J Boucher B. & Beaconsfield, P H rmon l anges during ribose-induced h poglycemi Diabetes 19 21, 1970
10. C 'talmann R & Illig, R Inability of g lactose t mobilize insulin in galactokinase-def cient indiv dual Diabetologia 5 143, 1969
11. Goetz, F C Maney J W & Greenberg, B Z The regulation f insulin secretion. Effects f th infusion f glucose ribose, and th sugars into the portal ein f dogs J Lab clin Med 69 537 1967
12. Goodman, C & Goetz, F C Oral and intravenous D-ribose nd plasma insulin in healthy humans Effects of route of dministration and f epinephrine and propranolol Metabolism 19 1094, 1970
13. Grodsky G M Batts, A. A Bennett, L. L., Vcella, C M Williams, N B & Smith, D F Effects of carbohydrates on secretion f insulin from isolated rat pancreas. Amer J Physiol 205 638, 1963
14. Halter J B Schwartz, M Z & Goetz, F C The effect of oral and intravenous D-ribose on plasma insulin levels in unanesthetized dogs. Proc Soc. exp Biol. Med 127 1147 1968
15. Hirata, Y F jisawa, M., Sato H., Asano, T & Katsuki, S. Blood glucose and plasma insulin responses t xylitol administrated intravenously in dogs Biochem. biophys Res. Comm. 24 471 1966.
16. Hirata, Y Fujisawa, M Sato, H., Asano, T & Katsuki, S. Effects of intravenous injection of xylitol on blood sugar blood pyruvic acid and plasma insulin levels in the dog. Z. Ges. exp. Med. 148 111 1968.
17. Hug, G & Schubert, W K. Serum insulin in Type I glycogenosis. Effect f galactose or fructose administration. Diabetes 16 791 1967
18. Kuzuya, T Kanazawa, U & Kosaka, K. Stimulation f insulin secretion by xylitol in dogs. Endocrinology 84 200, 1969
19. Lacy P E., Young, D A. & Fink, C. J Studies on insulin secretion in vitro from

isolated islets of the rat pancreas. Endocrinology 83 1185, 1968.

20. Lambert, A. E., Junod, A., Stauffacher W., Jeanrenaud, B. & Renold, A. E. Organ culture of fetal rat pancreas. I. Insulin release induced by caffeine and by sugars and some derivatives. Biochim. biophys. Acta 184 529 1969

21 Landgraf, R., Kotler Brajtburg, J & Matschinsky F.. Kinetics of insulin release from the perfused rat pancreas caused by glucose, glucosamine, and galactose. Proc. Nat. Acad. Sci. U.S.A. 68 536, 1971.

22. Lin, B. J & Haist, R. E. Insulin biosynthesis Effects of carbohydrates and related compounds. Canad. J Physiol. Pharmacol. 47 791, 1969

23. Malaisse, W & Malaisse-Lagae, F.. Effects of ribose and pentitols upon insulin secretion. Arch. Int. Physiol. Bioch. 77 2, 1969

24. Malaisse, W J Malaisse-Lagae, F & Brisson, G.. Combined effects of glucose and sulfonylureas on insulin secretion by the rat pancreas in vitro. In Recent hypoglycemic sulfonylureas U C. Dubach and A. Bückert, ed., 114, Hans Huber Bern, Switzerland, 1971

25. Malaisse W Malaisse-Lagae, F & Mahy M. Effets du mannose sur la sécrétion d'insuline. Ann. Endocrin. (Paris) 30 695 1969

26. Malaisse, W J Malaisse-Lagae, F & Mayhew D A possible role for the adenyl-cyclase system in insulin secretion. J clin. Invest. 46 1724, 1967

27 Malaisse, W J Malaisse-Lagae, F Walker M. O. & Lacy P E. The stimulus-secretion coupling of glucose-induced insulin release. V The participation of a microtubular microfilamentous system. Diabetes 20 25 1971

28. Malaisse, W Malaisse-Lagae, F & Wright, P H. A new method for the measurement in vitro f pancreatic insulin secretion. Endocrinology 80 99 1967

29 Malaisse-Lagae F Brisson, G R. & Malaisse W J The stimulus-secretion coupling of glucose-induced insulin release. VI. Analogy between the insulinotropic mechanisms of sugars and amino acids. Horm. metab. Res. 3 374, 1971

30. Malaisse-Lagae, F & Malaisse, W J The stimulus-secretion coupling f glucose-induced insulin release III Uptake of 45Calcium by *isolated islets f Langerhans Endocrinology* 88 72, 1971.

31 Marks, V & Samols, E. Action de différents stimuli (galactose, fructose et lipides) sur l'insulino-sécrétion humaine Influence du tractus intestinal. In Journées de Diabétologie de l'Hôtel Dieu 1969, 179 Flammarion, Paris, France, 1969

32. Matchinsky F M., Ellerman, J E., Krzanowski, J Kotler Brajtburg, J Landgraf, R. & Fertel, R. The dual function of glucose in islets of Langerhans. J biol. Chem. 246 1007 1971.

33. Modigliani, E., Strauch, G. & Odievre, M. Hormonal response to intravenous fructose in normal and fructosaemic children A study of insulin and growth hormone secretion. Rev Europ. Etudes Clin. et Biol. 15 882, 1970.

34. Montague, W & Taylor K. W Pentitols and insulin release by isolated rat islets of Langerhans. Biochem. J 109 333, 1968.

35 Nijjar M. S. & Perry W F Effects of intravenous and oral infusion of monosaccharides on serum insulin levels in rabbits. Diabetes 19 155, 1970

36. Pipeleers, D G Marichal, M. & Malaisse, W J Unpublished observations.

37 Rommel, K., Melani, F Burkhardt, H. & Grimmel, K.. Einfluß der Galaktose auf die Insulinsekretion beim Menschen. Diabetologia 5 309 1969

38. Sloviter H. A. & Petkovic, M. R.. Stimulation of insulin secretion in the rabbit by D-ribose. Nature 221 371 1969

39 Spitz, I. M., Rubenstein, A. M., Bersohn, I. & Bassler K. H. Metabolism of xylitol in healthy subjects and patients with renal disease. Metabolism 19 24, 1970.

40. Steinberg, T Ortman, P Poucher R., Cochran, B. Jr & Gwinup, G The effect of D-ribose infusion on serum immuno-reactive insulin and glucose concentration. Metabolism 16 40 1967

41. Sussman, K. E., Vaughan, G D & Timmer R. P An in vitro method for studying insulin secretion in the perfused isolated rat pancreas. Metabolism 15 466, 1966.

42. Telib, M. Vergleichende Untersuchungen über den Einfluss von Monosacchariden und Hormonen auf die Insulinsekretion des isolierten Pankreasgewebes einiger Säugetiere und des Frosches. Z. Ges. exp. Med. 147 316, 1968.

43. Thorell, J I. & Persson, B.. Transient stimulation of insulin release by fructose in newborn pigs. Endocrinology 86 897 1970.

44. Wilson, R. B & Martin, J M.. Plasma insulin concentrations in dogs and monkeys after xylitol, glucose or tolbutamide infusion. Diabetes 19 17 1970.

Acta med. scand. Suppl. 642, pp. 165—172

THE CORTICOID INSULIN AND GROWTH HORMONE RESPONSES TO INTRAVENOUS FRUCTOSE IN MEN AND WOMEN

J M. Aitken, D A. G Newton, P E Hall and A J Dinwoodie

From the Department of Medicine and Department of Biochemistry Western I firmary Glasgow and Regional Steroid Laboratory Royal I firmary Glasgow U K.

Abstract. The corticoid, insulin and growth hormone (HGH) responses to intravenous fructose were studied in 20 men and 12 healthy oophorectomized women, who were given an infusion of 100 mg/kg fructose over a period of five minutes Blood samples were obtained before the infusion and at intervals for one hour thereafter

Significant rises in plasma corticoid concentration were not observed, but significant falls appeared at one hour in all subjects Plasma insulin concentrations rose significantly within 10 minutes of the infusion and fell to sub-fasting values by one hour in the males, but similar changes were not found in the females. Fasting HGH concentrations were significantly higher in the females than in the males and t one hour after fructose these values fell in the former and rose in the latter to a significant degree.

In the men the changes in plasma insulin were directly related to the changes in plasma glucose. Changes in plasma corticoid and HGH concentrations were inversely related to the changes in plasma insulin.

In the women the above interrelationships were not demonstrable, but both the 30 minute changes in plasma glucose and insulin were found to be directly related to the degree of obesity

These responses to fructose in the men are similar to those induced by glucagon and it is suggested that the initial response to fructose may be the release of glucagon.

Some controversy has existed about the efficacy of fructose as a stimulus to insulin release. Kilo et al. (7) demonstrated insulin secretion in anaesthetised dogs who were given an unspecified quantity of fructose. Grodsky et al. (5) obtained only slight stimulation of insulin release in isolated rat pancreas in the presence of a fructose concentration of 500 mg/100 ml, whereas Coore and Randle (3) were unable to demonstrate any insulin secretion from isolated rabbit pancreas using fructose concentrations of 300 mg/100 ml. In 1969 we reported significant rises in plasma insulin concentration in healthy men after giving 31 Gm fructose intravenously over a period of five minutes (1), and later we described similar changes in a comparable group of men following acute myocardial infarction (2) In both these instances plasma fructose concentrations did not exceed 120 mg/100 ml.

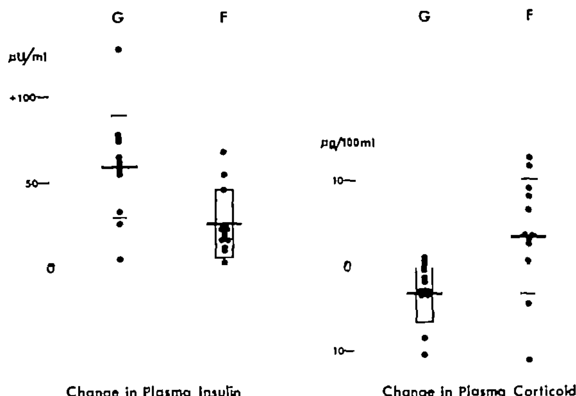


Fig 1 Chang in plasma insulin and corticoid concentrations 30 minutes after giving either 31 g glucose (G) or fructose (F) intravenously in 12 healthy men. Means ± 1 S D given.

In the normal subjects studied there was in addition a significant rise in plasma corticoid concentrations and in view of the frequent occurrence of abdominal pain after such large doses of fructose it seemed possible that the insulin response might have resulted from a reaction to stress and not as a direct response to fructose itself (Fig 1) This suspicion was supported by the finding that the insulin response and the rise in blood glucose after fructose were both directly related to the rise in plasma corticoid (Figs. 2 and 3)

In the present study we have examined the insulin, corticoid and growth hormone (HGH) responses to a much smaller intravenous bolus of fructose, which was found not to cause any physical discomfort whatever

MATERIAL AND METHODS

Twenty men with normal glucose tolerance (some of whom wer convalescent hospital in-patients, the others being healthy medical personnel) and twelve healthy middle-aged oophorectomized women (all of whom were outpatients) were studied. All subjects readily volunteered to participate in this study after the details of the test had been explained to them. Following a 10—12 hour overnight fast and batinence from smoking, each subject was given an infusion of 20 per cent fructose intravenously in a dose of 100 mg/kg over a period of five minutes. All out patients rested recumbent for t least 15 minutes prior to the test In ll subjects blood samples were btained fasting nd at 30 and 60 minutes after the start of the infusion. In eight men additional samples were obtained t 10 and 20 minutes. None of the volunteers experienced either abdominal or chest discomfort during or fter the fructose infusion.

Blood samples wer analysed for plasma glucose by glucose xidase procedure (9) using a standard Boehringer kit, and plasma corticoid was measured by fluorimetry (10), insulin and HGH were measured by radioimmunoassay the former using a Sephadex bound insulin antibody

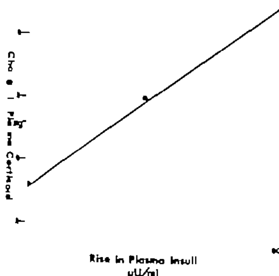


Fig 2 Relation between 30 minute insulin and corticoid change after 31 g fructose i.v in 12 healthy men (r = 0 68, t = 2 90 p < 0 02)

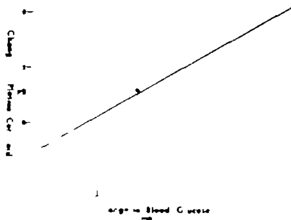


Fig 3 Relation between 30 minute glucose and corticoid change after 31 g fructose i v in 12 healthy men (r = 0 59 t = 2 29 p < 0 05)

(13) and the latter by a modification of the standard antibody/antigen precipitation method (6)

In the women obesity was assessed by measuring body weight and height and from this the Fat Score was derived, where

$$\text{Fat Score} = \sqrt[3]{\frac{\text{weight (kg)}}{\text{height (cm)}}} \times 1000$$

RESULTS

The baseline data on the subjects studied is given in Table I. The details of the response of these subjects to intravenous fructose are shown in Table II.

Plasma glucose concentrations rose significantly within 10 minutes of the infusion by a mean of 13 mg/100 ml ($t = 6\ 40$ $p < 0.001$), and remained significantly elevated until 30 minutes after the infusion, but by one hour the plasma glucose concentration was no longer significantly above fasting values. Similar changes were not seen in the women.

Mean plasma corticoid concentrations remained unchanged fo the first 30 minutes in the males, but by one hour there was a significant mean fall of 3 4 µg/100 ml ($t = 2.77$ $P < 0\ 01$). In the females the mean plasma corticoid concentrations had already

Table I *Basal values of subjects studied. Means ± SEM given*

	Men (20)	Women (12)
Age (range)	45 (24—65)	52 (47—57)
Weight kg	73 (± 2.3)	64 (± 2.9)
Fat Score	—	25 (± 0.5)
Plasma glucose mg/100 ml	83 (± 2.2)	86 (± 1.4)
Plasma corticoid µg/100 ml	21 (± 1.2)	18 (± 1.6)
Plasma insulin µU/ml	13 (± 1.8)	10 (± 1.9)
Plasma growth hormon µU/ml	2 (± 0 6)	8 (± 1.2)

Table II. *Response to i.v fructose 100 mg/kg in men and women. Means above fasting values given ± SEM Significant differences from fasting values given thus*

		Change from fasting values at			
		10 min	20 min	30 min	60 min
Plasma glucose mg/100 ml	Men	+ 12.9 ** (± 2.0)	+ 8.9 **** (± 1.6)	+ 8.5 **** (± 1.2)	+ 1 7 (± 1.8)
	Women	—	—	— 0.7 (± 2.2)	+ 0.2 (± 1.3)
Plasma corticoid μg/100 ml	Men	+ 1 4 (± 1.3)	+ 1.0 (± 1.6)	+ 0 4 (± 1.2)	— 3.4 ** (± 1.2)
	Women	—	—	— 3.1 ** (± 1.0)	— 4.8 *** (± 0.9)
Plasma insulin μU/ml	Men	+ 11.9 ** (± 2.8)	+ 5.9 ** (± 2.0)	+ 0.3 (± 1.0)	— 3.3 (± 1 4)
	Women	—	—	+ 0 4 (± 0 9)	+ 0 4 (± 0.6)
Plasma growth hormone μU/ml	Men	+ 0.1 (± 0.1)	+ 0.8 (± 0 4)	— 0.1 (± 0.5)	+ 1.3 † (± 0 7)
	Women	—	—	— 0.7 (± 1.2)	— 1.3 † (± 1.0)

$p < 0.05$ $p < 0.02$ ** $p < 0.01$ **** $p < 0.001$ † $p < 0.05$

fallen significantly by 30 minutes ($t = 3.22$, $p < 0.01$) and fell still further at one hour by a mean of 4.8 μg/100 ml ($t = 5.20$ $p < 0.001$)

In the men, plasma insulin concentrations rose by a mean of 12 μU/ml at 10 minutes, but by 30 minutes these had fallen to fasting values and at one hour the mean plasma insulin concentration was significantly below the fasting value by 3.3 μU/ml ($t = 2.32$, $p < 0.05$) In the women in whom measurements were only made at 30 and 60 minutes, no significant mean changes in plasma insulin concentration were found.

Mean fasting plasma HGH concentrations were significantly higher in the women at 8 μU/ml as compared with the men in whom the values was 2 μU/ml ($t = 4.61$ $p < 0.001$). In the latter there was a slight fall in the HGH concentrations at 30 minutes and this

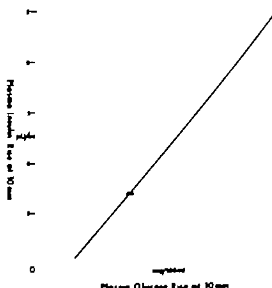


Fig 4 Relation between 10 minute insulin and glucose change after 100 mg/kg fructose i.v in 8 healthy men $y = 1.23x - 3.92$ ($r = 0.86$, $t = 4.10$ $p < 0.01$)

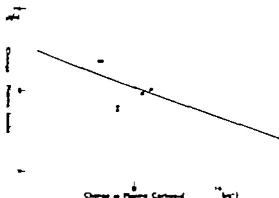


Fig 5 Relation between 30 minute insulin and corticoid change after 100 mg/kg fructose i.v. in 10 healthy men, $y = -0.30x + 0.38$ ($r = -0.46$ $t = 2.18$, $p < 0.05$).

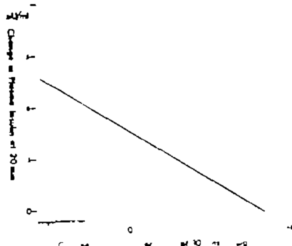


Fig 6 Relation between 20 minute insulin and 10 minute corticoid change after 100 mg/kg fructose in 8 healthy men $y = -1.22x + 7.61$ ($r = -0.82$ $t = 3.45$ $p < 0.02$)

was followed by a small rise at one hour whereas there was a small progressive fall at both 30 and 60 minutes in the women. The mean change in HGH concentration at one hour was $+1.3$ μU/ml in the men and -1.3 μU/ml in the women ($t = 2.20$ $p < 0.05$).

On comparing the plasma insulin and glucose changes at 10 minutes after the infusion in the males (Fig 4), a significant direct correlation was found ($r = 0.86$, $t = 4.10$ $p < 0.01$). No correlation was found between the plasma corticoid change and the corresponding change in plasma glucose at any time after the fructose infusion. The 30 minute plasma insulin and corticoid changes (Fig. 5) were inversely related ($r = -0.46$, $t = 2.18$, $p < 0.05$). Similarly on comparing the 10 minute corticoid change with the 20 minute insulin change (Fig 6) a much more significant inverse correlation was found ($r = 0.82$, $t = 3.45$ $p < 0.02$). Analogous comparisons between these parameters were not found in the female subjects, but the changes observed in this group were discovered to be related to the degree of obesity. The Fat Score, which is the same as the reciprocal of the Ponderal Index (in c.g.s.

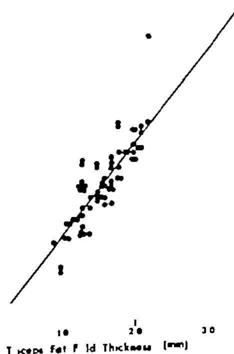


Fig. 7 Relation between Fat Score and Triceps Fat Fold Thickness in 106 middle-aged women $y = 0.23x + 20.68$ ($r = 0.82$, $t = 14.35$ $p < 0.0001$).

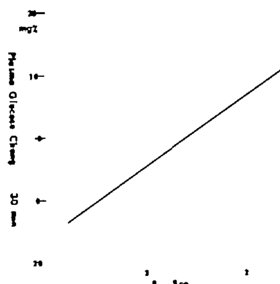


Fig 8 Relation between 30 minute plasma glucose change after 100 mg/kg fructose i.v and Fat Score in 12 healthy middle-aged women $y = 2.87x - 73.49$ (= 0.63, t = 2.59 $p < 0.05$)

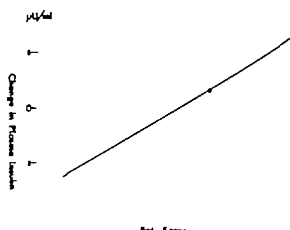


Fig 9 Relation between 30 minute plasma insulin change after 100 mg/kg fructose i.v and Fat Score in 12 healthy middle-aged women $y = 1.48x - 37.21$ ($r = 0.75$, $t = 3.61$ $p < 0.01$)

units) correlates well with the Triceps Fat Fold Thickness (in mm) in middle-aged women (Fig 7) When the Fat Score was compared with the change in plasma glucose at 30 minutes (Fig 8) a significant correlation was found ($r = 0.63$ $t = 2.59$ $p < 0.05$) Similarly the change in plasma insulin at 30 minutes was directly related to the Fat Score (Fig 9) to a significant degree ($r = 0.75$ $t = 3.61$ $p < 0.01$)

A significant inverse correlation was found between the insulin and HGH changes at 30 and 60 minutes (Fig 10) in the males ($r = 0.57$ $t = 3.96$ $p < 0.001$) A similar relationship was not found in the females.

DISCUSSION

The present study confirms beyond doubt that even relatively small intravenous doses of fructose in man are followed by significant rises in plasma insulin concentration The temporal relationship of insulin secretion after fructose infusion is similar to that seen after intravenous glucose in so far as in both instances the maximal rises occur within about 10 minutes of infusion. Although the true plasma glucose concentrations rose *pari passu* with the rises in plasma insulin and the magnitude of these responses were significantly correlated, this does not necessarily imply that the rise in plasma glucose *per se* caused the associated insulin secretion. Had the glucose rise caused the insulin secretion one might have expected to see a better correlation between the earliest glucose rise and the subsequent rise in plasma insulin concentration, but such a relationship was not found.

Stimulation of the pituitary adrenal axis by the fructose infusion was not demonstrable in this study and in fact plasma corticoid concentrations fell in the same way and to the same extent as have been demonstrated previously after glucose infusions (1). Hence in this instance stress could not be implicated as the mechanism responsible for either the rises in plasma glucose or plasma insulin concentrations.

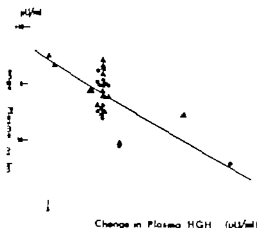


Fig 10 Relation between 30 minut (open tri angles) and 60 minute (closed circles) plasma insulin and HGH changes after 100 mg/kg fructose L in 20 healthy men y = —1.15x—1 48 (r = —0 67 t = 3.96, p < 0 001).

The lack of significant mean changes in plasma insulin concentrations after fructose infusion in the women studied was not surprising in view of the negligible changes at 30 minutes in the men. In the absence of blood samples taken at 10 and 20 minutes after infusion it should not be assumed that a similar pattern of insulin secretion after fructose occurred in the women. In view of the small range of insulin and glucose changes at 30 minutes in these subjects it could be argued that this merely represented randomly scattered values about zero Had this been true one would not have expected to find any relationship between the size or degree of obesity of the women studied and the associated changes in plasma insulin and glucose concentrations after fructose infusion. Since changes in both plasma glucose and insulin were directly related to the degree of obesity it seems likely that these plasma changes were not random variations but reflected a specific response to the fructose infused. It was interesting that the most obese women experienced the greatest glucose and insulin responses. If the magnitude of the glucose and insulin responses to fructose were dose related, as was suggested by Kilo et al. (8) one would have expected the heavier subjects to have shown the largest rises Hence the relationship between obesity and insulin response might have been an artefact since the most obese women tended also to be the heaviest and hence received most fructose. In this context no correlation was found between the insulin change at 30 minutes and body weight in either the women or the men studied. It should be remembered that since obese subjects have relatively less extra cellular fluid (ECF) they would tend to have somewhat higher ECF fructose concentrations than lean subjects in the immediate post infusion period Alternatively the heightened response to fructose found in the obese women could be the result of either an inherent metabolic difference peculiar to obesity or the release of a metabolite of fructose derived from its catabolism in adipose tissue. Both pyruvate and glycerol, which are intermediaries in the catabolism of fructose could have played a part in augmenting the insulin response to the small changes in plasma glucose (3 12) This aspect of the insulin response to fructose requires further investigation.

The differences in fasting HGH concentrations found between the males and females were similar to those reported by Frantz and Rabkin (4) In view of the similarity between the action of glucose and the present small dose of fructose in lowering plasma corticoid, it might have been expected that the usual HGH lowering effect of glucose would have been observed in this study after fructose. Whereas fructose infusion caused an initial mean fall in plasma HGH concentration in both groups, this was followed by a rise in the males at one hour — the response being significantly different from that seen in the females in whom the fall continued. Although these changes were small, the pattern was similar to that found after the s.c. injection of glucagon in men (11)

All the changes in the males reported in this study could be explained on the basis of an initial release of glucagon by the fructose infusion, but clearly this requires confirmation.

ACKNOWLEDGEMENTS

We are indebted to Dr Martin Gallacher for the growth hormone assays.

This work was supported by grants from the Scottish Hospital Endowments Research Trust and G D Searle Ltd., High Wycombe

REFERENCES

1. Aitken, J M. & Dunnigan, M. G Insulin and corticoid response to intravenous fructose in relation to glucose tolerance. Brit. med J 3 276 1969
2. Aitken, J M., Dunnigan, M. G & Ford, J A. Utilisation of glucose and fructose in acute myocardial infarction Proceedings of 2nd International Symposium on Clinical and Metabolic Aspects of Laevulose (1970)
3. Coore, H. G & Randle P J Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro Biochem. J 93 66, 1964
4. Frantz, A. G & Rabkin, M. T Effects of estrogen and sex difference on secretion of human growth hormone J clin. Endocr 25 14 0 1965.
5. Grodsky G M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N B. & Smith, D F Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Amer J Physiol. 205 638 1963.
6. Hunter W M. & Greenwood, F C. A radio-immunoelectrophoretic assay for human growth hormone. Biochem. J 91 43, 1964.
7. Kilo C., Long, C. L., Bailey R. M., Jr., Koch, M. B. & Recant, L. Studies to determine whether glucose must be metabolised to induce insulin release. J clin. Invest. 41 1372, 1962.
8. Kilo, C., Devrim, S., Bailey R. & Recant, L. Studies in vivo and in vitro of glucose-stimulated insulin release. The effects of metabolizable sugars, tolbutamide and 2-deoxyglucose. Diabetes 16 377 1967
9. Marks, V An improved glucose-oxidase method for determining blood, C. S. F and urine glucose levels. Clin. chim. Acta 4 395, 1959
10. Mattingly D A simple fluorimetric method for the estimation of free 11 hydroxycorticoids in human plasma. J clin. Path. 15 374, 1962.
11. Mitchell, M. L., Byrne, M. J & Silver J Growth hormone release by glucagon. Lancet 1 289 1969
12. Pelkonen, R., Miettinen, T A., Taskinen, M & Nikkilä, E. A. Effect of acute elevation of plasma glycerol, triglyceride and FFA levels on glucose utilization and plasma insulin. Diabetes 17 76, 1968
13. Wide, L. & Porath, J Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. Biochim. biophys. Acta 130 257 1966.

Acta med. scand Suppl 54., pp. 171—1 9

EFFECT OF SUCROSE FEEDING ON GLUCOSE TOLERAI CE

A. M. Cohen

From the Diabetic Unit, Hadassah U versity Hosp't a d Hebrew University, H dassah Medical School, J rus — l re

Abstract Feeding high sucrose diets t human volunteers and experimental animals results in impaired glucose tolerance, hyperlipidemia and impaired serum insulin-like activity Breeding selected rats with impaired glucose tolerance caused by sucrose feeding produces in succeeding generations. diabetes, renal arteriosclerosis, and renal and retinal diabetic like angiopathy The starch fed siblings develop none of these changes. The interaction between genetics and sucrose consumption in the production of diabetes mellitus is thus proved.

This experimental model explains the development of diabetes in Yemenite Jews immigrating to Israel. In the Yemen, where their diet contained no sucrose, the incidence of diabetes was ex tremely low After settling in Israel, where they consumed large amounts of sucrose, the incidence of diabetes has risen.

It was suggested by Claude Bernard in 1877 (2) that the organism s tolerance to carbohydrates is affected by previous nutritional factors. Himsworth (16) has elaborated on this and has shown that a high carbohydrate diet may improve glucose tolerance in normal persons. Uram et al. (24) suggested a hypoglycemic substance in starch.

The extreme change that has occurred in several Jewish ethnic groups as a result of immigrating to Israel, in their external environment, mode and habits of life and food habits offered the possibility to study the effect of these changes on the prevalence of diabetes.

In a field survey on the prevalence of diabetes in Israel, 16,000 people were ex amined. It was found that the prevalence of diabetes was 1 per cent for the Sephardi ethnic group and 2.5 per cent for the Ashkenazi ethnic group, with an average of 1.8 per cent for the total population (4) However the difference between the Ashkenazi and Sephardi groups was not significant. This incidence of diabetes among Jews in Israel is not greater than that among non-Jews in other parts of the world (1, 15 17 18, 21, 23, 25).

On examining about 5000 Yemenite new immigrants to Israel, we could find only 3 cases of diabetes, i.e. the prevalence was almost zero However on examining Yemenites who settled in the country for more than 25 years, the incidence of diabetes was

Table I *Food intake per capita per day*

Groups	No. of families	Calories	Protein Intake (g)	Fat: Animal & margarine	Fat: Oil	Carbohydrates: Total (g)	Carbohydrates: Sucrose (g)
Yemenites in Yemen	70	3[illegible] ± 98	60 ± 3 6	42 ± 3	14 ±	351 ± 15	6 6 ± 1 (1 18)
Yemenite old settlers	70	559 ± 118	86 ± 4 4	51 ± 3	30 ± 3	377 ± 31	63 ± 6 (9.9)
European old settlers	*19*	*2648 ± 1 1*	*80 ± 4*	*5 ± 4*	*33 ± 4*	*363 ± 33*	*9[illegible] ± 3 (12.9)*

Values are means ± SD
Figures in parentheses = % of total calories from sucrose

higher than that observed in the Ashkenazi Western Jew The same observation was repeated also among the Kurdish new immigrant Jews where no diabetes was found, while among the Kurdish old settlers, the incidence of diabetes was also higher than among the Ashkenazi Jews (4)

A dietary survey carried out with Dr Bavly and Mrs Poznanski among the Yemenites revealed that 1) in the Yemen most of the fat consumed was from animal sources and 2) almost no sucrose was consumed, while in Israel a high percentage of the carbohydrate intake was on the form of sucrose and a large percentage of the fat was in the form of unsaturated fat (Table I) (7). The possible association of this change in diet, i.e the increased sucrose consumption, and the increased incidence of diabetes was suggested by us in 1961 (5) Similar observations were made in 1963 in and around Durban, relating the increased incidence of diabetes in Asians and Africans with the increased sucrose consumption (3)

Accordingly in collaboration with Mrs. Teitelbaum two groups of albino rats were put on fully-supplied synthetic diets (Table II) in which the carbohydrate (72 per cent) consisted in the experimental group of sucrose and in the control group of corn starch. After keeping the animals for two months on these diets, oral glucose tolerance *was found to be impaired in the sucrose-fed* group. Similarly the insulin like activity of the sucrose-fed group was lower and the growth curve was found to be impaired when compared with that of the starch-fed group (Table III) (10) Reducing the protein content in the diet made these changes more severe (11) However the nitrogen balance in the sucrose-fed rats was not different from that of the starch fed rats (12)

In order to rule out the possibility that the impairment of the glucose tolerance was due to a change in the rate of glucose absorption from the gastrointestinal tract.

Table II. *Contents of diets (synthetic)*

	Percentage by weight		
	Starch	Sucrose	Glucose-Fructose
Vitamin-free casein	18	18	18
Butter	5	5	5
Starch	72	0	0
Sucrose	0	72	0
Glucose	0	0	36
Fructose	0	0	36
Salt and vitamins **	5	5	5

* USP salt mixture no. II.
** Containing 0.2 mg thiamin, 0.2 mg riboflavin, 0.1 mg pyridoxine, 1.6 mg calcium pantothenate, 5 mg niacin, and 100 mg cholin chloride per 100 g. Fat soluble vitamins A and D added twice weekly

intravenous glucose tolerance test was performed. The glucose removal rate (K) was calculated according to the equation.

$$K = \frac{2.3\ (\log Gt_1 - Gt_2)}{t_2 - t_1}$$

where Gt is the excess blood glucose concentration at time t and is equal to the blood glucose at time t minus the blood glucose fasting level. The value K represents the percentage removal of the excess glucose per minute

The glucose removal rate (K) from the blood (Table III) was significantly smaller in the sucrose-fed animals (0.03 ± 0.0022) than in the starch fed controls (0.040 ± 0.0028)

In order to clarify whether the impaired glucose tolerance is due to the sucrose molecule or to the glucose and fructose fractions, a group of albino rats were fed the same synthetic diet but the carbohydrate was supplied as 36 per cent fructose and 36 per cent glucose. The oral glucose tolerance performed after ... months feeding revealed that it is impaired as in that of the sucrose-fed animals (Table III)

Together with Groen (13) human volunteers were fed in alternate 5-weekly periods on diets composed of saturated fat, 40 grams proteins, 70—80 grams carbohydrate, 250—300 grams On changing the kind of *carbohydrate in each period from sucrose* to bread and *vice versa* we have shown that after the bread diet period the glucose tolerance test was lower than after the sucrose diet period The blood cholesterol showed a rise on the sucrose diet and a drop on the bread diet.

Table III. *Effect of diet on weight gain, blood glucose and insulin like activity of serum in rats*

Diet	No. of animals	Gain in body wt (g)	Blood glucose mg^a 60'	120'	P^b	K	P^b	Insulin-like activity mg glucose used 100 mg diaphragm	P^b
Starch	10	80 ± 4.7	120 ± 3.4	110 ± 2.8	—	0.040 ± 0.0028	—	0.750 ± 0.063	—
Sucrose	9	66 ± 5.9	150 ± 4.2	155 ± 3.5	0.05	0.030 ± 0.022	0.05	0.411 ± 0.071	0.01
Glucose + Fructose	10	72 ± 6.3	154 ± 5.1	162 ± 4.5	0.05	—	—	—	—

Values are means ± SE.
a After being fasted 16 hours, then given glucose load (350 mg/100 g body wt)
b Probability of difference between the respective groups and the starch group.
See text.

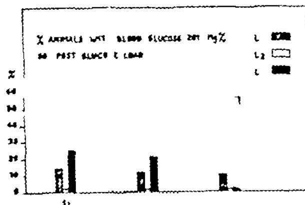


Fig 1 Blood glucose values at 60 minutes following an intragastric glucose load (350 mg/100 g body weight) in the selected generations (S_1 S_2, S_3) of the upward selected lines L_1 and L_3 and the downward selected line L_2.



Fig 2 Starch — fed rat. Normal glomerulus Hematoxylin and eosin, x420.

In collaboration with Mrs Saliternik (14) 28 male and 19 female albino rats from the animal house — parent generation (P) — at the age of 21 days, were fed on sucrose diet for two months. A the end of this period, a glucose tolerance test was performed. A male and two females wi h the highest blood glucose values were mated — *Upward Selection* — and the descendants of these couples called *Lines I and III* L kewise a male and a female from the P generation with the lowest blood glucose values were mated — Downward Selection — and the offspring called *Line II* The descendants of Lines I II and III were also fed on sucrose diet for two months ans similarly selected, Lines I and *III* upwards and Line II downwards. Fig. 1 shows the percentage of animals following an oral glucose load with blood glucose above 200 mg/100 ml in the different selected generations of Lines I III and II. It shows definitely that with further selec tion in Lines I and III (upward selection) the percentage of animals with blood glucose above 200 mg 100 ml increases while in Line II (downward selection) there is no difference in the succeeding selected generations

The rats fed high sucrose diet for long periods developed renal changes of the na-



Fig 3 Sucrose — fed rat. The glomerulus is enlarged and there is marked widening of the mesangial areas, hematoxylin and eosin, x420.

ture of diffuse glomerulosclerosis (Figs. 2 and 3) and in electron microscopy a thickening of the basement membrane-like material was observed. (Fig 4) (9 20) The trypsin digested retina showed loss of mural and endothelial cells irregularity of capillary diameter varicose capillary loops strand formation and microaneurysms (Figs. 5 and 6) (8)

These lesions in the kidneys and retina are identical with vascular lesions observed in spontaneous and experimentally induced

Fig. 4. Sucrose — fed rat. Electron microscopy of the glomerular mesangium. Note the increase of the mesangial matrix, the electron density of which is identical with the peripheral basement membrane. x22, 800

diabetes. Renal and retinal vascular changes were not observed in any of the starch-fed age-matched controls.

Preliminary studies in *Acomys cahirinus* revealed that sucrose feeding resulted in similar changes as noted in the albino rat, i.e. impaired glucose tolerance excessive rise in serum cholesterol and triglycerides and an increased enzymatic activity in the liver of enzymes involved in lipogenesis, glycolysis and gluconeogenesis.

It appears that the decisive factor for the glycemic response is the rate of glucose inflow into the circulation and stimulation of the insulin system. Normal rats fed stock

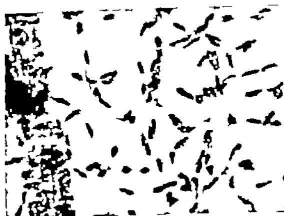


Fig. 5. Starch — fed rat. Digested flat retina (control) showing general normal appearance of the vessels. P.A.S. and hematoxylin, x420.

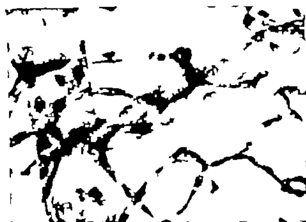


Fig 6 Sucrose — fed rat. Digested flat retina showing diminution of mural and endothelial cells and microaneurysm. P.A.S and hematoxylin, x420.

diet (Table IV) had a significantly higher blood glucose level response to intragastric sucrose load than to a starch load (0.5 g/100 g body weight) (10) Thus compared to starch, the absorption of glucose from the gastrointestinal tract in the case of sucrose feeding is rapid, leading to a steep post prandial rise in blood glucose with resulting stronger stimulation of the insulin system (22)

These and other experiments (19 24) show that high sucrose diet impairs the carbohydrate balance in both man and animals, as compared to animals fed dietary carbohydrates in the form of starch, while keeping constant in other dietary ingredients. Furthermore, vascular complications characteristic of diabetes have been found in animals fed sucrose.

The genetic study in animals has shown the interaction between the genetic factor(s) and the dietary changes in producing diabetes. This is a laboratory duplication of what has been observed in nature among some ethnic groups with a specific pattern — the Yemenite and Kurdish new immigrants to Israel and the Bantus in South Africa — in whom the incidence of diabetes has risen with the change in their environment, and the change in their diet, consisting mainly of an increase in sucrose consumption. We have shown that there is an individual variation in the degree of carbohydrate metabolism impairment resulting from feeding on a high sucrose diet (6)

The necessity for the interaction between the genetic and dietetic factors in the development of diabetes, as demonstrated in the above experiment, explains why only a certain percentage of people develop diabetes on consuming large amounts of sucrose. Furthermore, the necessity for a long feeding period for the development of diabetes may explain in part why the incidence of diabetes rises with age.

Table IV *Effect of intragastric loads of sucrose and starch, 0.5 g/100 g body wt., on the glucose blood level of normal rats fed stock diet*

Load	No of animals	Fasting blood glucose, mg/100 ml	Excess blood glucose, mg/100 ml			
			30'	60'	90'	120'
Corn starch	10	67.2 ± [illegible]1	28 ± 2.8	29 ± 2.9	24 ± 2.9	14 ± 2.3
Sucrose	9	66 1 ± 1 2	53 ± 1.9	76 ± 2.0	61 ± 1.9	62 ± 5 1

Values are mean ± SE.

REFERENCES

1. Albertson, V Diabetes in Iceland. Diabetes 2: 184, 1953.
2. Bernard, C. Lecons sur le diabète et la glycogenèse animale. Paris, Baillière, 1877 p. 70.
3. Campbell, G D Diabetes in non-white persons in Southern Africa. S. Afr med. J 37 1195 1963.
4. Cohen, A. M. Prevalence of diabetes among different ethnic Jewish groups in Israel Metabolism 10 50 1961.
5. Cohen A. M. Fats and carbohydrates in atherosclerosis and diabetes in Yemenite Jews. Am. Heart J 65 291 1963.
6. Cohen, A. M. Effect of dietary carbohydrate on the glucose tolerance curve in the normal and the carbohydrate-induced hyperlipemic subject. Am. J clin. Nutr 20 126, 1967
7. Cohen, A. M., Bavly S. & Poznanski, R Change of diet of Yemenite Jews in relation to diabetes and ischemic heart disease. Lancet 2 1399, 1961.
8. Cohen, A. M., Michaelson, I. C. & Yanko, L. Retinopathy in rats with disturbed carbohydrate metabolism following a high sucrose diet Part I. Vascular changes. Am. J Ophth., 73 863, 1972.
9. Cohen, A. M. & Rosenmann, E. Diffuse glomerulosclerosis in sucrose-fed rats. Diabetologia 7 25, 1971.
10. Cohen, A. M. & Teitelbaum, A. Effect f dietary sucrose and starch on oral glucose tolerance and insulin like activity Am. J Physiol. 206 105, 1964.
11. Cohen, A. M. & Teitelbaum, A. Effect of different levels of protein on sucrose and starch diets on lipid synthesis in the rat. Israel. J med. Sci. 2 727 1966.
12. Cohen, A. M. & Teitelbaum, A. Effect of different levels of protein in »sucrose« and »starch« diets on the glucose tolerance and growth. Metabolism 15 1034, 1966.
13. Cohen, A. M., Teitelbaum, A., Balogh, M. & Groen, J J Effect f interchanging bread nd sucrose a main source of carbohydrate in low f t diet on the glucose tolerance curve f h althy lunteer subjects Am. J clin. N t 19 59 1966
14. Cohen, A M T [illegible] lbaum A & Saliternik, R [illegible] tics d diet as fact rs in th development [illegible] d [illegible] mellitus Metabolism 21 [illegible]
15. G [illegible] M & Vassterstorm S S A pilot [illegible] w York [illegible] J Med [illegible]
16. [illegible] P [illegible] ph [illegible] Sci [illegible]
17. [illegible] & Ch [illegible] A [illegible] D [illegible] Diabetes [illegible]
18. L [illegible] 15 [illegible]
19. [illegible] Som [illegible] f dietary [illegible] and depot [illegible] J [illegible] 18 334, 196[illegible]
20. Rosenmann E Teitelbaum, A & Cohen A M [illegible] path in sucrose fed rat Electron d Light mi oscopi studies Diabetes 20 803 19 1
21. Schliack, Diabetesprobleme Untersuchungen ber di Haufigkeit, den Altersaufbau, das Manifestationsalter und Manifestationsbedingungen des Diabetes Mellitus Dtsch. med Wschr 79 855, 1954
22. Swan, D C Davidson, P & Albrink, M J Effect of simpl and complex carbohydrat on plasma non-esterified fatty acids, plasma sugar and plasma insulin during ral carbohydrate tolerance test. Lancet 1 60, 1966.
23. Tulloch, J A. & Johnson, H. M. Pilot survey of the incidence of diabetes in Jamaica. W Ind med J 7 134, 1948.
24. Uram, J A Friedman, L. & Kline, O L. Influence of diet on glucose tolerance Am. J Physiol. 192 521, 1958.
25. Wright, H. B. & Taylor B The incidence of diabetes in sample of the adult population in South Trinidad. W Ind. med. J 7 123, 1948.

ready in 1874 that diabetic patients were able to utilize fructose given orally better than other sugars (12)

Several studies have shown that the rate of fructose elimination from the blood is the same in the diabetic as in the normal man. Liver vein catheterization studies have shown the same rate of fructose uptake in the splanchnic area in healthy subjects as in diabetic patients (6) Minkowski, in 1893 observed that fructose in contrast to glucose, led to glycogen formation in the liver when given to pancreatectomized dogs (16) However the effects of fructose and glucose infusions on the glycogen formation in liver and muscle have not earlier been studied systematically by direct technique in diabetic man. We have therefore made a series of experiments, trying to elucidate these problems, and some preliminary results are presented here. Corresponding studies in normal man have earlier been performed in our laboratory (2, 17)

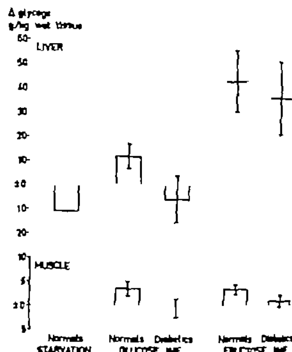


Fig 1. Changes (mean ± SD) in liver and muscle glycogen after i.v fructose and glucose infusions (1 g/kg b.w./4 hrs) to normals and diabetics. The columns denote the changes from the values after an overnight fast. For comparison the mean decrease of the liver glycogen content after corresponding 4 hrs' period of starvation in normals is given.

EXPERIMENTAL PROCEDURES

Insulin was withdrawn f m the insulin-dependent patients 36 hours before the experiment. After an overnight fast glucose or fructose solutions were given intravenously to the subjects at a rate of 1 g per kg body-weight per hour A control group of normal subjects was treated in the same manner

Percutaneous li er and muscle biopsies were performed before nd fter the infusion, and the glycogen content was determined in the tissue samples (10) Glucose lactate pyruvate, nd ketone bodies in blood were determined at intervals in all cases In addition, the changes in the fructose concentration and in the acid-base balance in blood were followed during the fructose infusions

RESULTS

Fructose infusion caused an increase of the liver glycogen content amounting to 35 5 g per kg wet weight. The increase was of the same magnitude in the normal subjects as in the diabetic patients. The muscle glycogen increase in normal subjects after fructose infusion was 3.3 g/kg as a mean (3) However in contrast to the findings in the normals, the muscle glycogen content in the diabetics showed no significant change after fructose (Fig 1)

Glucose infusion caused a significant increase of the liver glycogen content in normal subjects of 11.2 g/kg liver Contrarily to the increase of the liver glycogen in the normals, no change of the liver glycogen was observed in the diabetics in spite of very high blood glucose concentrations (500—1 000 mg per 100 ml) In the normal subjects the administration of glucose increased the muscle glycogen by 3.5 g/kg The muscle glycogen level in the diabetic patients remained unchanged after glucose (Fig 1)

The mean fructose level in blood during fructose infusion was in the normals 90 mg/100 ml and in the diabetics 120 mg/100 ml. The elevation of glucose in blood during fructose infusion was small in the normals but considerable in the diabetics. These observations are in agreement with previous studies (6)

Blood lactate increased rapidly and the increase was of the same magnitude in normals and in diabetics during fructose in fusion. Concomitantly with the rise in blood lactate there was a fall in standard bicar bonate and pH in blood.

A rapid decrease of β-hydroxybutyrate and acetoacetate concentration in blood was observed both in the normals and the dia betics during fructose infusion.

Fructose excretion in urine was small and did not exceed 5 per cent of the infused quantity in diabetics or normal subjects. Fructose increased the urine glucose excretion in some of the diabetics but no glucosuria was found in the normals.

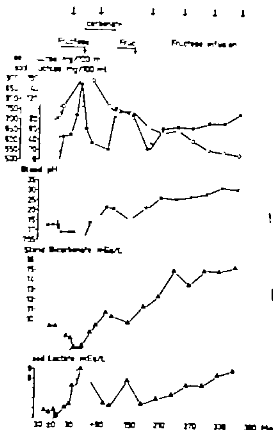


Fig 2 Fructose infusion to patient with severe keto-acidosis. The first fructose infusion was discontinued because of increasing acidosis. Insulin and bicarbonate were given i.v. A continuous infusion of fructose could not be given till after repeated doses of insulin. In spite of insulin administration during the last fructose infusion blood lactate increased.

At each ↓ 20 units of insulin was given i.v.

DISCUSSION

Several advantages for the clinical use of fructose have previously been presented (15 24). Some of these advantages are shortly summarized in the following Fructose is metabolized without the presence of insulin. Fructose has a large distribution space and is rapidly metabolized, resulting in low blood concentration and a small urinary loss of the carbohydrate. Fructose decreases the plasma free fatty acids (16) The rapid increase of the respiratory quotient during fructose infusion, observed by many authors, can at least partly be due to a release of CO_2 from body fluids by the lactic acid formed during fructose metabolism. The protein sparing effect of fructose is a result of decreased gluconeogenesis from amino acids.

Complications due to fructose infusion have been reported As mentioned before part of the fructose infused is transformed to lactic acid in the liver (2). Lactic acid gives rise to an acidosis, the degree of which is directly correlated to the infusion rate In uncon trolled ketotic diabetics we have observed an aggravation of the condition of the patients, caused by the addition of a lactic acidosis to the keto-acidosis (Fig. 2) (3). The decrease of the bicarbonate concentration in blood through lactic acid formation from fructose was considerable. Insulin in combination with infusion of sodium bicarbonate in large

quantities may be necessary to improve this severe condition. The risk of provoking a lactic acidosis is a limitation for the use of fructose in diabetics with manifest or latent acidosis.

Liver and muscle glycogen are main stores of carbohydrate in the body The formation of glycogen in the diabetic state after fructose or glucose infusion is still a controversial problem. There are three main steps for the transformation of hexose to glycogen in liver and muscle, which can be of interest for the discussion, i.e. the penetration of the sugar through the cell membrane the phosphorylation, and the transformation from UDP-glucose to glycogen.

1 Glucose penetrates the liver cell independent of insulin while the penetration into the muscle cell is insulin-dependent (13) Fructose is not dependent on insulin for its penetration into liver or muscle (6)

2. The phosphorylation of glucose is brought about by hexokinase in liver and muscle and glucose is also phosphorylated liver by a specific enzyme, glucokinase, hich is insulin-dependent for its synthesis (5) The fructose phosphorylation in muscle by hexokinase is inhibited by glucose at normal concentrations according to data from animal experiments but evidence for a direct phosphorylation in human muscle of fructose under *in vivo* conditions have been presented (7). In the liver fructose is phosphorylated also by a specific ketohexokinase which is not dependent on insulin action (1) and not inhibited by glucose

3 The transformation of UDP-glucose to glycogen is mediated by glycogen synthetase which appears in an active form (I) and an inactive form (D) (19) We have found the I activity and the total activity (I + D) to be reduced in muscle of untreated diabetic patients (*0) No similar studies have as yet been made in human liver tissue In normal experimental animals a conversion of the D to the I form has been demonstrated in liver and in muscle after administration of glucose or of insulin (25) *In vitro* studies with fructose gave no D to I conversion in contrast to glucose (8)

According to our studies in untreated diabetic patients no increase of either synthetase I activity or glycogen synthesis occurs in muscle during glucose infusion. After insulin administration a conversion of the D to the I form is found concomitantly with a rise of glycogen (21) Similar results are reported by Villar Palasi et al. from studies in vivo of alloxan-diabetic rats (25). The liver of alloxan-diabetic rats has low activity of the I form, which does not increase during refeeding a carbohydrate rich diet that causes a substantial increase of the glycogen content (9) It has previously been suggested that glycogen synthetase is the rate-limiting enzyme for glycogen synthesis and that only the I form is active (7 19). The D form is inhibited by physiological concentrations of ATP and inorganic phosphate (19)

The lack of glycogen formation in liver after glucose infusion in the diabetics can be explained by a low glucokinase and/or hexokinase activity or possibly by a low conversion of synthetase D to I. It is not probable that glycogen synthetase is the rate-limiting factor as the liver glycogen content in the diabetics was normal before the infusion. The block in glycogen synthesis from glucose is therefore more likely to be at the site of glucose phosphorylation.

In muscle already the entry of glucose into the cell is slowed by a defective penetration, which — like the enzymes involved in glycogen synthesis — is insulin-dependent (14).

The rapid formation of glycogen from fructose in diabetic liver indicates a normal activity of fructokinase and a sufficient synthetase activity for normal glycogen formation.

A pronounced decrease in ATP level due to fructose phosphorylation (11) could de-inhibit the synthetase D

ACKNOWLEDGEMENTS

This investigation was supported by grants from the City of Stockholm, the Swedish Medical Research Council (projects nos B71 19X 1002-06 and B71 19X 2647-03) the Swedish Nutrition Foundation, the Semper Foundation for Nutrition Research nd the Swedish Life Insurance Companies' Council for Medical Research.

REFERENCES

1. Adelman, R. C., Ballard, F J & Weinhouse, S Purification and properties of rat liver fructokinase. J biol. Chem. 242 3360 1967
2. Bergström, J & Hultman, E. Synthesis of muscle glycogen in man after glucose and fructose infusion. Acta med. scand. 182 93, 1967
3. Bergström, J Hultman, E. & Roch-Norlund, A. E. Lactic acid accumulation in connection with fructose infusion. Acta med scand. 184 359, 1968.
4. Bondy P K., Bloom, W L, Whitner V S. & Farrar B. W Studies of the role of the liver in human carbohydrate metabolism by the venous catheter technic. II. Patients with diabetic ketosis, before and after the administration of insulin. J clin Invest. 28 1126, 1949
5. Borrebaek, B Hultman, E Nilsson, L. H:son, Roch-Norlund, A. E. & Spydevold, O Adaptable glucokinase activity of human liver Biochem. Med. 4 469, 1970
6. Craig, J W Drucker W R Miller M., Owens, J E., Woodward, H., Brofman, B. & Pritchard, W H. Metabolism of fructose by the liver of diabetic and non-diabetic subjects. Proc. Soc. exp Biol (N Y) 78 698, 1951.
7. Danforth, W H Glycogen synthetase activity in skeletal muscle. Interconversion of the two forms and control of glycogen synthesis. J biol. Chem. 240 588, 1965
8. Hers, H. G De Wulf, H Stalmans, W & Van den Berghe, G The control of glycogen synthesis in the liver Adv Enzyme Regul. 8 171, 1970.
9. Hornbrook, R. Synthesis of liver glycogen in starved alloxan diabetic rats Diabetes 19 916, 1970
10. Hultman, E Muscle glycogen in man determined in needle biopsy specimens. Method and normal values Scand J clin Lab Invest. 19 209 1967
11. Kekomäki, M Raivio, K. O & Mäenpää, P H Interference with liver metabolism by D-fructose. Acta anaesth. scand. Suppl. 37 114, 1970
12. Kulz, E. Beitrage zur Pathologie und Therapie des Diabetes Mellitus. Marburg, 1874
13. Levine, R., Goldstein, M Klein, S. & Huddlestun, B. The action of insulin on the distribution of galactose in eviscerated nephrectomized dogs. J biol. Chem. 170 985 1949
14. Malette, L. E., Exton, J H. & Park, C. R. Control of gluconeogenesis from amino acids in perfused rat liver J biol. Chem. 244 5713, 1969
15. Miller M., Craig, J W Drucker W R. & Woodward, R The metabolism of fructose in man. Yale J Biol. Med. 29 335, 1956
16. Minkowski, O. Untersuchungen über den Diabetes Mellitus nach Extirpation des Pancreas. Arch exp. Pathol. Pharmakol. 31 85, 1893.
17. Nilsson, L. H:son & Hultman, E. Liver glycogen in man. The effect of total starvation, different diets and of glucose and fructose infusions. IV World Congr Gastroenterology Copenhagen, 12—18 July 1970
18. Opitz, K The influence of xylitol and other polyols and sugars on fat mobilization. p. 238 in Pentoses and Pentitols. Eds B L. Horecker K. Lang & Y Takagi, Springer Verlag Berlin, Heidelberg, New York, 1969.
19. Piras, R Rothman, L. B. & Cabib, E. Regulation of muscle glycogen synthetase by metabolites. Differential effects on the I and D forms. Biochemistry 7 56, 1968.
20. Roch-Norlund, A. E.. Muscle glycogen synthetase in patients with diabetes mellitus. Basal values, effect of glycogen depletion by exercise, and effect of treatment. Scand. J clin. Lab. Invest. 29 237 1972.
21. Roch-Norlund, A. E., Bergström, J & Hultman, E. Muscle glycogen synthetase in normal subjects and in patients with diabetes mellitus. Scand. J clin. Lab. Invest. In press.
22. Roch Norlund, A. E., Bergstrom, J Castenfors, H. & Hultman, E. Muscle glycogen content in patients with diabetes mellitus. Glycogen content before treatment and the effect of insulin. Acta med. scand. 187 445 1970
23. Stetten, De W Jr The study of certain patologic processes with the aid of isotopic hydrogen. N. Y St. J Med. 47 1991 1947

24 Thorén, L. Parenteral nutrition with carbohydrate and alcohol. Acta chir scand. Suppl. 325 75, 1964.
25. Villar Palasi, C., Goldberg, N O Bishop, J S., Nuttall, F Q & Larner J Metabolic action and enzyme regulation. FEBS Symposium 19 149 1969
26. Wahren, J The role of the liver in diabetes and prediabetes. Presented at the Medicinsk Riksstämma, Stockholm, Nov 1971.

Acta med. scand. Suppl. 542, pp 187—193

METABOLIC EFFECTS OF DIETARY FRUCTOSE IN INSULIN DEPENDENT DIABETES OF ADULTS

Risto Pelkonen, Antti Aro and Esko A. Nikkilä

From the Third Department of Medicine, University of Helsinki, 00290 Helsinki 29, Finland

Abstract. The metabolic effect of an isocaloric replacement of 75 grams of dietary starch by fructose was studied in 10 insulin-dependent diabetic patients. Body weight, urinary excretion of glucose, diurnal variation of blood glucose, plasma free fatty acids (FFA) and plasma immunoreactive growth hormone (IRGH) and fasting levels of plasma cholesterol and triglyceride were determined during three successive dietary periods. During the first and third period starch was the main carbohydrate given while during the second period 75 grams of starch was replaced by fructose. Fructose feeding did not alter diurnal blood glucose or urinary output of glucose but a tendency to an increase of plasma triglyceride and FFA levels was observed during the fructose period. Plasma levels of cholesterol and IRGH were unaffected by fructose.

The results suggest that in controlled insulin-dependent diabetes moderate amounts of fructose can be included in the diet without any adverse effects on the glucose balance. However it seems likely that triglyceride metabolism of diabetics is influenced by dietary fructose even in moderate doses.

Since the observations, one hundred years ago that in diabetic organism fructose is utilized better than glucose many investigations have been carried out to evaluate the position of dietary fructose in the management of clinical diabetes (6, 18, 20, 14, for review see 7). There are, however only few controlled studies where the metabolic effects of fructose and glucose have been compared (6, 18). The results showed that in mild and moderate diabetes isocaloric replacement of dietary glucose by a reasonable amount of fructose (up to 90 g) diminishes hyperglycemia and glucosuria. This beneficial effect of fructose is not seen in more severe form of diabetes, however. The general conclusion of all these experiments has been that fructose as a therapeutic agent does not have any place in the treatment of clinical diabetes.

As pure fructose has now been made commercially available as a nutrient it was of interest to study whether insulin dependent diabetic patients are able to use moderate amounts of this sugar without worsening of glucose or lipid metabolism.

MATERIAL AND METHODS

The dietary experiments were carried out in ten (five males and five females) insulin dependent

diabetics selected from patients attending the Diabetes Outpatient Unit of the University Central Hospital of Helsinki. The median age was 25.5 years (range 19 to 70) and the relative body weight was within 90 to 118 per cent (mean 99). In all subjects the diabetes was of moderate degree the daily dose of insulin being from 22 to 84 units (mean 41) and the disease being relatively stable if not always well con trolled with this dosage. The mean duration of the disease was 12.4 years (range 3 to 22). Seven patients had some signs of diabetic complications but none had uremia (serum creatinine normal) or severe vascular disease.

All patients were taken t the metabolic ward for the whole study period. On admittance each was put on sugar free hospital diet the amount of which was adjusted to become isocaloric. Insulin was given as single injection of long acting preparation (Insulin Lente® Novo or Insulin Rapitard Novo) each morning and the dose was adjusted to give the best possible steady state condition in regard of body weight, blood glucose and urinary glucose output. It should be noted that a strict control of the diabetes was not attempted but just a stable condition with minimum day-to-day variations. One patient needed two daily injections of Insulin Lente® The subjects were allowed a normal physical activity in hospital but no extra strenuous exercise

After the dosage f insulin and body weight had been stabilized the dietary experiment was started. This consisted of three periods, ten days each. During the first and third periods the diet contained starch as the major carbohydrate (designed STARCH I and II) while during the second period 75 grams f starch was exchanged for the same amount of fructose (FRUCTOSE). In one patient the second starch period had to be omitted. The general outline of the study is shown in Fig. 1

Starch I and II The diet during these periods was identical. It provided a total mount of 30 to 40 calories per kg per day and the pproximate distribution f these was 40 per cent from carbohydrate 40 per cent from fat (mainly saturated) and 20 per cent from protein. The carbohydrate was mainly starch the total amount of oligosaccharides (lactose sucrose fructose) being bout 25 grams daily. The diet was given as six meals between 8 m and 9 p m

Fructos During this period 5 grams of fructose wa added to the starch diet by sim l taneously decreasing the calculated amount of starch to maintain the total carbohydrate intak

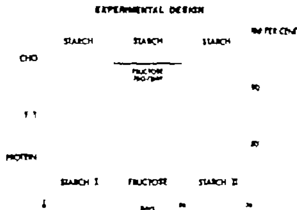


Fig 1 Arrangement of dietary periods.

constant. The fructose covered thus an average amount of 40 per cent of carbohydrate calories and pproximately 18 per cent of the total energy supply. Fructose was administered in five portions during the meals any single dose never exceeding 20 grams.

The following parameters were used as indicators of diabetic control

Daily urinary excretion of glucose The quantitative excretion of glucose was determined by polariscope every day

Diurnal level of blood glucose The diurnal variation of blood glucose was determined every third day by measuring the level of blood glucose every fourth hour during 24 hours beginning from 8 a.m. The patients were fasting from 9 p.m. to 8 a.m. and thus the eight o'clock morning values represent the fasting blood glucose.

Diurnal level of plasma free fatty acids (FFA): The diurnal variation of plasma FFA (28) was determined simultaneously with blood glucose in five patients. In the other five patients fasting level of plasma FFA (at 8 a.m. after overnight fasting) was determined every third day

Diurnal level of plasma imm noreactive growth hormon (IRGH). The diurnal level of plasma IRGH (29) was determined simultaneously with the 4 h blood glucose curves in three patients.

Plasma cholesterol and triglyceride Fasting levels (fter over night fasting) of plasma cholesterol (24) and triglyceride (23) were determined every third day in eight patients.

Body weight Body weight was measured every day

Urinary ketone bodies K tonuria was assessed by Acetest reagets in each sample f urine containing glucose

Table I. *Urinary output of glucose, fasting blood glucose and body weight (mean ± S.E.) in 10 insulin dependent patients during three successive dietary periods*

	Starch I	Fructose	Starch II
Urinary glucose g/day	17.3 ± 5.2	16.7 ± 4.5	12.9 ± 3.2
Fasting blood glucose mmol/L	7.9 ± 0.6	8.6 ± 0.8	7.7 ± 0.7
Body weight kg	60.6 ± 2.5	59.5 ± 2.5	59.5 ± 2.4

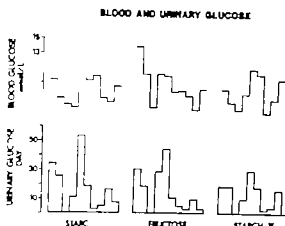


Fig. 2. Mean excretion of urinary glucose and mean levels of fasting blood glucose in ten insulin dependent patients during three successive dietary periods

Diurnal level of serum fructose (3) was also measured simultaneously with blood glucose in three patients.

For fasting blood glucose, FFA, cholesterol and triglyceride the mean values were calculated for each patient and dietary period. The results are presented as mean values of all patient means ± s.e.m. for each of the three periods.

RESULTS

All patients tolerated the fructose well without developing any side effects. Thus, none suffered from diarrhea, which often occurs with higher daily intakes of monosaccharides obviously as an osmotic effect. Most patients did not like the sweet taste of fructose. In spite of attempts to keep the diet at isocaloric level some patients reduced weight during the three experimental periods and the mean fall of 1.1 kg per 30 days occurred as is apparent from Table I. The insulin dosage remained constant through all three dietary periods in all subjects except one, in which reduction by 4 units was necessary during fructose period because of morning hypoglycemia.

Blood fructose. The levels of fructose in peripheral venous blood were too low to be accurately determined by the method used. The order of magnitude was 5 mg/100 ml or less and in none of the single determinations carried out from samples taken around the day did fructose concentration exceed a value of 10 mg/100 ml.

Glucose balance. The mean values of fasting blood glucose and of daily urinary excretion of glucose during the three dietary periods are presented in Table I. It is seen that all values were closely similar and there was no statistical difference in these parameters between the fructose and starch periods. Fructose feeding did not influence the fasting blood glucose or urinary glucose irrespective of whether the diabetic control was excellent or poor in the beginning of the experiment (Fig. 2). Thus, in patients with less strict control before the experiment the glucose status was not essentially worsened during the fructose period. Ketonuria was detected only in a few instances and there was no difference between the periods in this respect.

The diurnal blood glucose curves are presented in Fig. 3. All three curves are virtually identical indicating that fructose did not influence the diurnal variations or mean level of blood glucose.

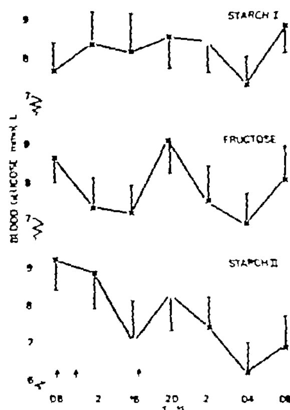


Fig. 3. Diurnal variation of blood glucose (mean ± S.E.) in ten insulin dependent diabetic patients during three successive dietary periods. The arrows indicate meals.

Table II. *Fasting levels of plasma cholesterol and triglyceride (mean ± S.E.) in eight and plasma FFA (mean ± S.E.) in ten insulin dependent diabetic patients during three successive dietary periods*

	Starch I	Fructose	Starch II
Cholesterol (mmol/L)	4.99 ± 0.1	5.10 ± 0.11	5.26 ± 0.13
Triglyceride (mmol/L)	1.00 ± 0.06	1.22 ± 0.08	1.05 ± 0.14
FFA (mmol/L)	0.61 ± 0.03	0.71 ± 0.04	0.60 ± 0.03

$p < 0.05$

Lipid metabolism. All patients had plasma cholesterol and triglyceride levels within normal range at the start of the dietary experiments. The mean values of these lipids as well as of fasting plasma FFA during the three periods are presented in Table II. Cholesterol level did not show any definite trend during fructose period as compared to starch but plasma triglyceride concentration tended to increase on fructose even though the difference between the means of fructose and starch periods is not significant. The fasting plasma FFA during fructose feeding was significantly ($p < 0.05$) higher than during any of the two starch periods. On the other hand, the diurnal variations of plasma FFA determined in five patients were not essentially different during the three dietary periods (Fig. 4). During fructose there was a tendency to lower plasma FFA after noon and night values as compared to starch but these differences were not significant.

Plasma growth hormone (IRGH). The mean diurnal level of plasma IRGH determined in three patients was very similar during the three dietary periods (Table III). The content of plasma IRGH did not fluctuate more during the fructose than during the two starch periods.

DISCUSSION

The metabolic pathway of fructose is in many respects dissimilar to that of glucose. The main bulk of ingested fructose is taken up by the liver and converted to trioses (15). A part of the ingested fructose is either converted to glucose in the intestinal wall, or taken up by adipose and muscle tissues (4, 5, 2, 17). The quantitative distribution of fructose assimilation between the various tissues is, however, not exactly known. Rapid metabolism of fructose to triose units occurs also in a diabetic liver (16, 17). The subsequent fate of trioses generated from fructose in diabetic organism apparently depends on the degree of insulin deficiency. If gluconeogenesis dominates as in severe diabetes increased production of glucose is the result while in controlled diabetes fructose is con-

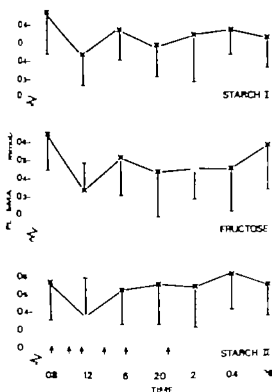


Fig. 4. Diurnal variation of plasma FFA (mean ± S.D.) in five insulin dependent diabetic patients during three successive dietary periods. The arrows indicate meals.

verted to pyruvate and other products of Krebs cycle. Since diabetic organism is capable for utilizing pyruvate fructose can really provide fuel to energy metabolism without increase of blood glucose (27).

In the present investigation the diabetes was in all patients at least moderately controlled, the mean excretion of urinary glucose varied from 0 to 53.7 g/day and the mean levels of fasting blood glucose varied from 5.6 to 10.9 mmol/L and none of the patients was ketotic. Therefore it was not unexpected that under these conditions replacement of 75 grams of dietary starch by an equal amount of fructose and given in small portions did not influence blood or urinary glucose. The results obtained are in concordance with the earlier observations that small amounts of fructose do not impair the diabetic control (6, 14, 18, 30).

Table III *The mean 24 hour-content of plasma IRGH (mean ± S.E.) in three insulin dependent diabetic patients during three successive dietary periods*

Diet period	Plasma IRGH ng/ml
Starch I	3.28 ± 0.58
Starch II	3.66 ± 0.55
Fructose	3.68 ± 0.62

The effect of dietary fructose on plasma lipids is important when the use of fructose as a dietary component of diabetic patients is considered. In experimental animals fructose exerts a more pronounced hyperglyceridemic effect than glucose (1, 20, 31). Moreover oral fructose enhances exogenous hyperglyceridemia in rat and man (21, 22). The hyperlipidemic effects of isocaloric exchange of various dietary carbohydrates (starch, sucrose, malt, glucose, fructose) are on the other hand somewhat contradictory as has been reviewed recently by Nikkilä (9—13, 19, 25). No previous data are available on the effect of fructose feeding on plasma lipids in diabetic man, however. It is obvious that insulin deficiency, whether corrected by exogenous insulin or not, modifies the interaction of carbohydrate feeding and lipid metabolism since the insulogenic stimulus exerted by the ingested carbohydrates is lacking. In the present investigation the tendency to develop hyperglyceridemia is noteworthy since the duration of the dietary periods may have been too short for an effective induction of hyperglyceridemia by the relatively small doses of fructose used. Furthermore, it is possible that the slightly negative caloric balance present in many cases masked to some extent the fructose effect.

A somewhat unexpected finding was the increase of fasting plasma FFA during the fructose period. No adequate explanation can

be offered for this phenomenon so far. In nondiabetics this could be attributed to the lesser stimulation of insulin secretion by fructose and concomitant increase of lipid mobilization from adipose tissue but the present diabetic patients were all receiving a constant dose of exogenous insulin during all three periods. The possibility of morning hypoglycemia and associated catecholamine response is excluded by the close similarity of the diurnal blood glucose curves during the fructose and starch periods. Since the elevated basal FFA level during fructose-rich diet may form one additional factor for the development of hypertriglyceridemia it should be of interest to confirm the present finding by a more extensive study including also nondiabetic subjects.

There were two reasons to include the plasma IRGH determinations into the present study. Firstly it has been shown that in diabetic patients plasma IRGH level is higher during poor than during good control (8) and, secondly because intravenous fructose has recently been postulated to stimulate growth hormone release (26). In the present experiments however that small oral doses of fructose did not influence the plasma level of IRGH.

The data accumulated in this investigation thus revealed that in short term experimental conditions isocaloric replacement of dietary starch by 73 grams of fructose does not influence the diabetic control as judged by the levels of blood and urinary glucose. The results suggest that the most critical point in the chronic use of fructose in diet can be the effect of fructose on lipid metabolism.

ACKNOWLEDGEMENTS

This investigation was supported by grant from Finnish Food Research Foundation. We are very grateful to Marja-Riitta T kinen, M.D. for the analyses of growth hormone, to Miss Mary Puomio, responsible nurse of the metabolic ward, for careful dietary and nursing control of the subjects and to Mrs. Annell Ollus, M. Agr. Sci. (nutr.) dietitian of the hospital, for calculation of the diets.

REFERENCES

1. Bar-On, H. & Stein, Y. Effect of glucose and fructose administration on lipid metabolism in rat. J. Nutr. 94 95 1968.
2. Bergström, J. & Hultman, E. Synthesis of muscle glycogen in man after glucose and fructose infusion. Acta med. scand. 182 93, 1967.
3. Brown, W. L., Kendall Young Jr. M. A. & Seralle, L. G. Simultaneous measurement of glucose and fructose. J. Lab. clin. Med. 49 630 1957.
4. Butterfield, W. J. H., Sargeant, B. M., & Whichelow, M. J. The metabolism of human forearm tissues after ingestion of glucose, fructose, or liquid glucose. Lancet 1 574, 1964.
5. Froesch, E. R. Fructose metabolism in adipose tissue from normal and diabetic rats. In. Handbook of Physiology. Section 5. Adipose tissue (Ed. by A. E. Renold and G. F. Cahill, Jr.) p. 282, American Physiological Society Washington, D. C.
6. Hiller, J. Die Laevuloseverwertung des adipösen Diabetes mellitus. I Mitteilung. Zur Analyse der Acetonurie und der Glucosurie. Ztschr. klin. Med. 133 388, 1933.
7. Huttunen, J. K. Fructose in medicine. A review with particular reference to diabetes mellitus. Postgrad. Med. J. 47 654, 1971.
8. Johansen, K. & Hansen, Aa. P. Diurnal serum growth hormone levels in poorly and well-controlled juvenile diabetics. Diabetes 20 239 1971.
9. Kaufmann, N. A., Gutman, A., Barzilai, D., Eshcar, J. Blondheim, S. H. & Stein, Y. Hypertriglyceridemia induced by dietary fat or carbohydrate and by uncontrolled diabetes. Israel J. med. Sci. 1 389, 1965.
10. Kaufmann, N. A., Poznanski, R., Blondheim, S. H. & Stein, Y. Changes in serum lipid levels of hyperlipemic patients following the feeding of starch, sucrose and glucose. Amer. J. clin. Nutr. 18 261 1966.
11. Lees, R. S. The plasma lipid response to two types of dietary carbohydrate. Clin. Res. 13 549 1965.
12. Macdonald, I. The effects of various dietary carbohydrates on the serum lipids during a five-day regimen. Clin. Sci. 29 193 1965.

13. Macdonald, I. Influence f fructose and glucose on serum lipid levels in men and pre- and postmenopausal women. Amer J clin. Nutr 18 369 1966.

14. Mehnert, H., Mahrhofer E. & Förster H. Indikationen und Kontraindikationen für die Verabreichung von Fructose und Diabetiker Münch. med. Wchnschr 106 193, 1964.

15. Mendeloff, A. I. & Weichselbaum, T E. Rol of the human liver in the assimilation of intravenously administered fructose. Metabolism 2 450, 1953.

16. Metz, R., Mako, M., Stevens, T & Franklin, J The metabolism of fructose in diabetes mellitus. J lab. clin. Med. 69 494, 1967

17. Miller M., Craig, J W Drucker W R. & Woodward, H. Jr.. The metabolism of fructose in man. Yale J Biol. Med. 29 335, 1956

18. Moorhouse, J A. & Kark, R. M. Fructose and diabetes. Amer J Med. 23 46 1957

19. Nikkilä, E. A.. Control of plasma and liver triglyceride kinetics by carbohydrat metabolism and insulin. Adv Lipid Res. 7 111 1969

20. Nikkilä, E. A. & Ojala, K. Induction f hyperglyceridemia by fructose in the rat. Lif Sci. 4 937 1965.

21. Nikkilä, E. A. & Ojala, K. Acute effects of fructose and glucose on the concentration and removal rate of plasma triglyceride. Life Sci. 5 89 1966.

22. Nikkilä, E. A. & Pelkonen, R. Enhancement of alimentary hyperglyceridemia by fructose and glycerol in man. Proc. Soc. exp. Biol. Med. 123 91, 1966.

23. Noble R. P & Campbell, F M.. Improved accuracy in automated fluorometric determinati of plasma triglycerides. Clin. Chem. 16 1 5 1970

24. Pe rsc S., Stern, S. & McGavack, T A curate method for the determination t al serum cholesterol. Analyt. Chem. 25 813 1953

25. Porte, D Jr Bierman, E. L. & Bagdade, J D S bstitution of dietary starch for dextrose in hyperlipemic subjects. Proc. Soc. exp Biol. Med. 123 814, 1966.

26. Strauch, G Pandos, P & Bricaire, P Fructose induced growth hormone release. J clin. Endocrinol. 32 582, 1971.

27. Takanami, A Ohya, S. & Goto, Y Urinary excretion of pyruvic acid citric acid and alpha-ketoglutaric acid after intravenous injection of pyruvate and fructose in diabetic patients. Tohoku J exp. Med. 72 163, 1960.

28. Trout, D L., Estes, E. H. J & Friedberg, S. J Titration of free fatty acids of plasma study of current methods and a new modification. J Lipid Res. 1 199 1960.

29. Wide, L. Radioimmunoassays employing immunosorbents. Acta endocrinol. 63 suppl. 142. 207 1969

30. Voss, G Über di Fruktosetoleranz bei Diabetes mellitus. Ärztl. Wschr 12 827 1957

31. Zakim, D Pardini, R. S., Herman, R. H. & Sauberlich, H. E. Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver Biochem. biophys. Acta 144 242, 1967

Acta med. scand. Suppl. 542, pp 195—202

DOES DIETARY FRUCTOSE AFFECT THE CONTROL OF DIABETES IN CHILDREN?

H. K. Åkerblom, Irmeli Siltanen and Anna Kaarina Kallio

From the Children's Hospital, University of Helsinki, Helsinki, Finland

Abstract. The purpose of the study was to find out whether fructose can be used as a dietetic sweetener for diabetic children.

Acute effects of ingested fructose were studied in 28 hospitalized diabetic children by giving fructose, 1.0 g/kg, at breakfast, and by comparing 3 hours postcibal blood glucose to that of control days, when a corresponding amount of calories was given as a regular breakfast. Postcibal hyperglycemia from 30 to 120 min was significantly less on fructose days as compared with control days. The differences between blood glucose on control and fructose days were significantly greater from 30 to 120 min than the chance variations on two control days.

The effects of more prolonged use of dietary fructose on diabetes were studied in 16 patients at home for four weeks by alternating between one week periods on diets which included 1.5 g/kg/day of fructose and control weeks of regular isocaloric sugar free diets. Glucosuria, recorded twice daily by Clinitest and once a week by measuring 24-hour glucose excretion, was similar during control and fructose weeks. Ketonuria was infrequent during either diet. Fructose did not elevate serum triglycerides.

Because dietary fructose, given in substantially larger amounts did not impair the control of diabetes, it is concluded that fructose can be used by diabetic children at a rate of 0.5 g/kg/day without negative effect so long as the patient otherwise adheres to a quantitative diet, includes fructose on an isocaloric basis, and only patient' mother administers the compound.

The fate of infused or ingested fructose in the diabetic organism has long aroused interest. Particularly the tolerance to dietary fructose has been the subject of numerous studies, ever since the early report by Külz (10) of a decrease in diabetics' glucosuria after incorporation of fructose in the diet.

However the fructose tolerance of diabetic children has not been sufficiently investigated so far. To explore whether dietary fructose affects the control of diabetes in children, controlled experiments were carried out. In one part of the study patients were given fructose as an acute load at breakfast, and, in another part, fructose was administered throughout the day during two one-week periods. The effect of fructose on the diabetic state was judged in the former group from postcibal blood glucose changes, and in the latter group from glucosuria and ketonuria.

Table I. *Composition of breakfast given to 26 diabetic children on control and fructose days*

Control day(s). Regular breakfast for diabetics		Fructose day Breakfast containing fructose 1.0 g/kg/body wt	
Porridge	100—150 g	Chocolate	10 g
		+ Cream	10—15 g
Milk	100—150 g	or Lemon juice	20—30 g
Bread	10— 15 g	Bread	10—15 g
Butter	2.5— 7 g	Butter	2.5— 7 g
Meat (veal)	20— 40 g	Meat (veal)	20—45 g
Orange juice	0—100 g	Egg (boiled)	one
CHO	45 *	CHO	45 *
Fat	35	Fat	35—37
Protein	20 *	Protein	20—18
Kcal, mean ± SEM	403 ± 16	Kcal, mean ± SEM	416 ± 12

20 * of total daily calories

PATIENTS AND METHODS

The data were obtained from 51 diabetic children. The patients (23 boys, 28 girls) ranged in age from 2 to 16 years, the mean age being 10 years. They were treated with intermediate-acting insulin, given once or twice daily. Fructose was given either as an acute load, 1.0 g/kg body weight at breakfast (series A) or in an amount of 1.5 g/kg body weight/day divided into several portions throughout the day during two one-week periods (series B).

Series A. Twenty-six diabetic children, hospitalized either because of newly detected diabetes or due to poor control of previously diagnosed disease were given fructose 1.0 g/kg body weight *at breakfast. The experiments were done in the* cases with recent onset (14 children) at the end of the initial stabilization period shortly before discharge and in the cases which had been admitted due to poor control (1_ children), after some improvement had been achieved. The composition of the isocaloric breakfast on control and fructose days is given in Table I. Fructose was included in chocolate or lemon juice. On the fructose days the majority of calories provided by carbohydrates in the breakfast came from fructose.

The diet during the rest of the day was an ordinary diet for diabetic children. The fructose day was either the day following two control days or day between them. The dose and time of administration (30 min before breakfast) of insulin remained unchanged for each patient during the control and fructose days. Capillary blood specimens for blood glucose determination were taken immediately before breakfast and 30, 60, 90, 120 and 180 min. after it. The mean value of the two control days for each sampling was used in the comparison with the fructose day value. In seven cases we have data from one control and one fructose day. Forenoon snack was combined with breakfast, and therefore the caloric content of the latter rose to 20 per cent of that of the day (Table I). The last blood specimen was taken before the patients had their luncheon. Blood glucose was measured by a commercial glucose oxidase method (Kabi Reagens, Stockholm). Blood fructose, lactate and pH on the fructose day were determined in five and serum triglycerides (0, 60 and 120 min) in six patients. Fructose was analysed by an anthrone procedure (4), and the interference of blood glucose was eliminated by correction. Lactate was determined by a commercial lactate dehydrogenase method (Biochemica Boehringer Mannheim), pH by the Astrup micro technique and triglycerides fluorometrically (16,9).

Series B. The children were studied at home during a period of 28 consecutive days. All patients had had diabetes for more than six months and they had passed the partial remission. The diet during the first and third weeks (control) was regular diet for diabetic children sugarfree 1000 kcal + 100 kcal/year daily and proportion of calories derived from carbohydrate, fat and protein were 45, 35 and 20 per cent, respectively. The diet during the second and fourth weeks (fructose) was isocaloric with the

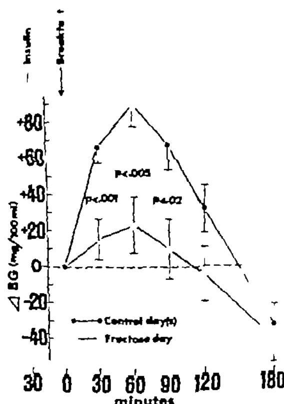


Fig. 1 Changes in blood glucose (mg/100 ml) mean ± SEM after a regular breakfast for diabetics, and an isocaloric fructose-containing breakfast in 26 diabetic children. Fructose dose 1.0 g/kg/ body wt.

diet of the control weeks, but a part of the carbohydrates from starch was substituted for by fructose at a rate of 1.5 g/kg/day and corresponding t about 20 per cent of the daily carbohydrates. Fructose was administered in beverage (10 g/ 100 ml) which the children drank t meals and snacks. The actual daily intake of carbohydrates was si lar from 203 ± 8 (mean ± SE) t 215 ± 9 g, d ring both types of weeks. The patients' mother received diet lists for each day of the observation period, sheets for recording the actual food intake, and were instructed in detail n dietary matters before the study. The insulin dose of each patient remained constant during the study and they were advised to exercise regularly and to the same extent during control and fructose weeks. The patients and/ or their mothers were checked t the start of the stud as regards their ability to do Clinitest and Ketostix tests properly. One of the investigators made home calls during the experimental period, re-checked the urine testing and discussed the dietary instructions. Clinitest® K tostix® tests were made every morning the patient's second morning urine specimen, ı he evening before bed time snack. For t ent the mean value of the daily Clinitest gs for each one week period was com- morning and evening specimens taken At the end of each week 24-hour urine mens were collected, brought to the laborato- rudied for glucosuria by polarimetry and t and for ketonuria by Ketostix®. Several had to be omitted from the series be- oncurrent acute infections, and a few t difficulties in adhering to diet prescrip- t or to inaccurate urine testing. Data on] ubjects are reported. Fasting venous blood s ples were drawn from some of these children t the end of each week for the determination of serum triglycerides.

The significance of differences between mean alues was calculated by Student's *t* test, and between individual observations by paired com parison. The differences in blood glucose values in series A between control and fructose days from corresponding specimens were compared to the chance variations of blood glucose of the same hour on two control days by the matched pair Wilcoxon test (3).

RESULTS

Series A.

Blood glucose (BG). Mean changes in BG after breakfast on control and fructose days are shown in Fig 1.

A paired comparison showed smaller postcibal increases in BG values on fructose day compared to control days from 30 to 120 min. ($p < 0.01$ < 0.001 < 0.005 and < 0.05 at 30, 60, 90 and 120 min. respectively). It is well known that in diabetic children BG at a certain time of the day may vary considerably from day to day. In this study however the differences between BG values on control and fructose days were significantly greater from 30 to 120 min. than the variations on two control days.

When the BG results of the subjects with recent onset of diabetes and of those ad mitted due to poor control were studied

Table II. *Changes in blood glucose (mean ± SEM) after a regular breakfast for diabetics, and an isocaloric fructose-containing breakfast in 14 diabetic children, admitted due to onset of the disease*

Fructose dose 1.0 g/kg body wt

	Δ 30 min	Δ 60 min	Δ 90 min mg/100 ml	Δ 120 min	Δ 180 min
Control day(s)	59 ± 11	80 ± 19	50 ± 21	8 ± 20	—56 ± 20
Fructose day	19 ± 17	28 ± 23	12 ± 25	—7 ± 22	—45 ± 18

Table III. *Changes in blood glucose (mean ± SEM) after a regular breakfast for diabetics, and an isocaloric fructose-containing breakfast in 12 diabetic children, admitted because of poor control of the disease*

Fructose dose 1.0 g/kg body wt

	Δ 30 min	Δ 60 min	Δ 90 min mg/100 ml	Δ 120 min	Δ 180 min
Control day(s)	73 ± 14	102 ± 15	87 ± 14	60 ± 14	— 7 ± 10
Fructose day	11 ± 15	19 ± 22	8 ± 23	—2 ± 22[b]	—37 ± 19

$p < 0.01$ vs control day(s)
[b] $p < 0.05$ — —

separately significantly lower BG increases after breakfast on fructose days compared to control days were observed only in the latter group of patients (Tables II and III).

The mean 0-values did not differ from each other on control and fructose days in either group of diabetics In cases with recent onset BG at 0 min. was 169 ± 13 (SE) on control and 163 ± 20 mg/100 ml on fructose days, and in cases with previously diagnosed diabetes 185 ± 20 on control and 207 ± .5 mg/100 ml on fructose days.

Blood fructose The mean blood fructose levels before and after a fructose-containing breakfast in five diabetics are given in Table IV

Blood lactate and pH Mean blood lactate values of five patients are seen in Table IV Hyperlactatemia paralleled the rise in blood fructose Blood pH remained unchanged after fructose ingestion in the same subjects despite the rise in blood lactate

Serum triglycerides Levels of serum triglycerides in the six patients studied were unaffected by fructose load. The mean value was 1.15 ± 0.31 (SE) at 0 min., 1.10 ± 0.27 at 60 min., and 1.16 ± 0.26 mmol/l at 120 min.

Series B

Glucosuria. The average concentration of glucose in urine measured at home by Clinitest® from morning and evening specimens is shown in Fig. 2.

Relative glucosuria was virtually identical during control and fructose weeks. Quantitative glucose excretion at the end of each week is given in Table V

Differences in glucosuria were found neither between group mean values during control and fructose weeks, nor in an individual paired comparison. Clinitest® recordings done in the laboratory on the 24-hr urine specimens were generally in good agreement with the results obtained with polarimetry

Table IV *Blood fructose and lactate (mean ± SE) [illegible] fruct -containing breakfast in five diabetic children*

Fructose dose 1 0 g/kg body wt

	0	30 min	6[illegible]	[illegible]	1[illegible] min	120 min
Fructose, mg/100 ml	3 2 ± 0 2	13.4 ± 1.8	8 8 [illegible]	[illegible]	5 ± 0 0	4 ± 0 4
Lactate, mg/100 ml	9.2 ± 1 0	28 0 ± 6.3	[illegible]	[illegible]	14[illegible] ± [illegible]	1 3 ± 1 4

a $p < 0.001$ vs 0
b $p < 0.005$
c $p < 0.02$
d $p < 0.05$

Table V *Glucosuria at the end of each wee[illegible] receiving dietary fructose 1.5 g/kg/day every [illegible]*

Diets were isocaloric during control and fructo[illegible]

	I week Control n = 15	II we[illegible] Fruc[illegible] n = [illegible]	[illegible]	I[illegible] F[illegible]
		Glucosur[illegible], g, [illegible] ± SEM		
8 a.m.—8 p.m.	24.2 ± 6.5	18.3 ± 5 0	[illegible]2 ± [illegible]	[illegible]
8 p.m.—8 a.m.	15.5 ± 3.9	16 9 ± [illegible] (14)	[illegible] ± [illegible]	[illegible]
Per 24 hours	39 7 ± 8 6	36.7 ± 9 3	[illegible]34 ± 1 6	[illegible]

Ketonuria. Ketonuria, as measured by Ketostix® was infrequent during both types of diets A few patients only had one positive recording out of 16 specimens analysed per week. A 2 year old boy had 5/16 positive recordings during the first control week, and thereafter 1/16 positive recordings during the subsequent fructose and control weeks.

The control of diabetes in the patients of series B before the trial with dietary fructose was on an average only fair as seen from the mean Clinitest® recordings of 1.2 and 1.5 % during the first week (Fig 2) and the average glucosuria of approximately 40 g/24 hr at the end of the first week (Table V).

Serum triglycerides. Fasting serum triglycerides, (mean ± SE, mmol/l), at the end of each week were as follows first week (control) 1.02 ± 0.10 (n = 7) second week (fructose) 0.91 ± 0.08 (n = 7) third week (control) 0.88 ± 0.05 (n = 9) and fourth week (fructose) 0.82 ± 0 07 (n = 6). Thu[illegible] no inc[illegible]rease occurred

Side effects

One diabetic in series B had diarrhea after drinking the fructose-containing beverage This ceased when the patient was given fructose in desserts and bakery Several children in both series stated that the fructose drinks tasted too sweet.

DISCUSSION

Fructose is absorbed from the small intestine in linear relation to the concentration of the sugar in the intestine (5) Although human mucosal cells contain the enzymes necessary for the conversion of fructose to glucose, such a transformation during the absorption through the intestinal wall in man is considered unlikely (5) or occurs only to a slight degree (3 14).

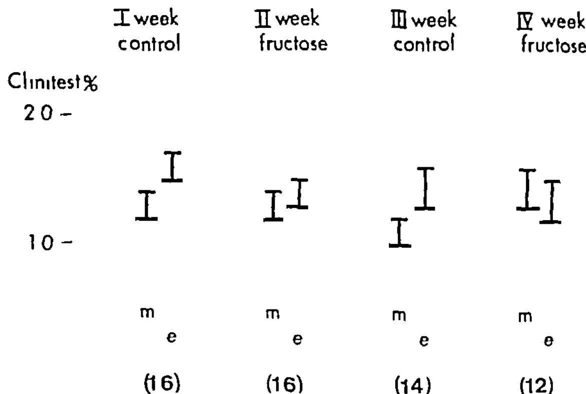


Fig   Relative glucosuria (%), mean ± SEM as measured by Clinitest® at home from morning (m) and evening ( ) specimens f diabeti children receiving dietary fructose 1.5 g/kg day every second week. Diets were isocaloric during control and fructose weeks. The number of subjects is indicated in brackets

In the liver fructose is rapidly metabolized to triose level by insulin-independent enzymes (20) The liver of diabetics can take up fructose without difficulty even in severe insulin deficiency (14) From the trioses the path goes either to glycolysis or to gluconeogenesis glyceraldehyde is metabolized to lactic acid, and dihydroxyacetone phosphate can be metabolized via the Embden—Meyerhof pathway to glucose and glycogen. Tracer studies indicate that a significant proportion of fructose entering the li er is converted to glucose in normal man (1)

Fructose-1 6-diphosphatase is one of the key-enzymes in gluconeogenesis (17) Its activity is increased in diabetes during insulin deficiency contributing to augmented gluconeogenesis from different precursors. The unimpaired hepatic uptake of fructose may therefore, result in an increased hepatic release of glucose. Some clinical reports on the intravenous or oral use of fructose in uncontrolled diabetes confirm the accelerated formation of glucose from fructose (6, 13 15) Relative insulin deficiency occurs in childhood diabetes at times despite treatment, and therefore fructose administration to these patients might theoretically lead to impaired control of the disease

Fructose has been widely used in some countries as a substantial constituent of the diet or as a sweetening agent by adult diabetics without deterioration of the control of the disease as reviewed by Mehnert (17). The daily dose has usually been 60—90 g or less, and the postulated advantages are slow absorption, utilization without insulin and antiketogenic effect. The slighter the diabetic state the greater are the benefits of fructose administration (12).

Only a few reports seem to be available on the effect of dietary fructose on the control of diabetes in children. Hartmann et al. (7) gave about half of the daily carbohydrates as fructose to five children for several weeks to see if an insulin-sparing effect would appear as compared to isocaloric glucose or sucrose feeding. The patients were in good or fair control during the study. The authors found no insulin-sparing effec of dietary fructose. Rosenkranz and Wagner (18) gave an acute fructose load of 1.5 g kg to nine children after the morning insulin and one hour after breakfast. The BG valu s ranged between 120 and 250 mg/100 ml before fructose administration. A decrease in BG was seen in eight patients within 90 min.

The present study was made to find out whether or not fructose could be used as a sweetener of food for diabetic children. For sweetening of food considerably smaller doses of fructose than we employed in either part of the investigation are sufficient. The fairly large doses of fructose were chosen to make it easier to reveal effects, if any on the parameters used as criteria for the control of diabetes.

The significant differences in the acute experiment between postcibal BG changes on fructose and control days (Fig 1) are difficult to explain. Fructose is absorbed slower than glucose at low concentration, whereas at a high concentration fructose and glucose are absorbed to a similar degree (8). Because the fructose concentration of the fructose day breakfast was high, fructose was presumably absorbed more rapidly than was glucose which was derived from slowly digested starch on control days. We cannot therefore attribute the milder hyperglycemia on fructose days to slow absorption. The diabetes of our subjects in series A was on the average under fair control, as seen from the mean BG levels at 0 min. (half an hour after the administration of insulin) on the experimental days. The difference between fructo nd con rol d ys in pos c ba BG ri e was great r i the pa ients ho h d been n b o p cor rol o e d [illegible] th [illegible]

[illegible] pa [illegible] sc [illegible] tr [illegible] this [illegible] than [illegible] var ati ns in ex rcise, em [illegible] up [illegible] may occas [illegible] influence t [illegible] and the result. Secondly the main parameter daily glucosuria, was measured in a way that was feasible for so long an observation period, but not ideal. Hence we regard our results on glucosuria measured at home (Fig 2) only as estimates. Because of these considerations minor effects of dietary fructose in one direction or another might not have been revealed. Nevertheless we cannot explain the different responses to fructose in series A and B.

Dietary fructose elevates serum triglycerides in nondiabetic men and post menopausal women (11). Fructose ingestion did not raise the serum triglycerides of our diabetic subjects, and particularly not in the cases of two boys who had reached puberty.

Our results do not give an answer to the question of the tolerance of dietary fructose, if consumed for months or years. In older literature the opinion prevailed that a long lasting use of fructose would lead to impaired utilization in diabetics in the course of time. Contrary to this assumption, Schar-

tow (19) found no change in the fructose tolerance of 89 adult diabetics, who took fructose 3 × 10 g daily for 18 months.

Because the diabetic state was not impaired in either part of our investigation, we conclude that in diabetic children, whose disease is in good or fair control, fructose can be used, at least for short periods, as one alternative for sweetening of food (desserts, bakery etc.) provided that

- The child adheres to a quantitative diet.
- Fructose is used on isocaloric basis.
- Only the child's mother administers the compound.

If fructose is used, the mother should be carefully instructed about the necessary calculations for the diet. For sweetening purposes, less than 1 g for instance 0.5 g/kg body weight/day is sufficient.

ACKNOWLEDGEMENTS

The study was supported by The Finnish Sugar Ltd.

REFERENCES

1. Atwell, M. E. & Waterhouse C. Glucose production from fructose. Diabetes 20 193, 1971
2. Bliss, C. I. Statistics in biology. Vol. I., p. 225. McGraw Hill Book Comp. New York 1967
3. Cook, G. C. Absorption products of d fructose in man. Clin. Sci. 37 675, 1969
4. Davidson, W. D. & Sackner M. A. Simplification of the anthrone method for the determination of inulin in clearance studies. J. Lab. clin. Med. 62 351 1963
5. Dawson, A. M. Carbohydrate absorption. Sci. Basis Med. 1969 p. 186
6. Felber J. P. Renold, A. E. & Zahnd, G. R. The comparative metabolism of glucose, fructose, galactose and sorbitol in normal subjects and in disease states. Mod. Probl. Pädiat. 4 467 1959
7. Hartmann, A. F. Sr. Wohltmann, H. J. & Hartmann, A. F. Jr. Dietary fructose in children with diabetes mellitus. J. Pediat. 45 27 1954.
8. Holdsworth, C. D. & Dawson, A. M. The absorption of monosaccharides in man. Clin. Sci. 27 371 1964.
9. Kessler G. & Lederer H. Fluorometric measurement of triglycerides. In Automation in analytical chemistry (ed. L. T. Skeggs, Jr.), p. 341. Ed. Mediad, New York 1965.
10. Külz, E. Beiträge zur Pathologie und Therapie des Diabetes Mellitus. Vol. I, p. 130. Marburg 1874.
11. Macdonald, I. Influence of fructose and glucose on serum lipid levels in men and pre- and postmenopausal women. Amer. J. clin. Nutr. 18 369 1966.
12. Mehnert, H. Die Verwendung von Fructose, Sorbit und Xylit. In Handbuch des Diabetes mellitus. Band II (ed. E. F. Pfeiffer) p. 1069. J. F. Lehmanns Verlag, München 1971.
13. Metz, R., Mako, M., Stevens, T. & Franklin, J. The metabolism of fructose in diabetes mellitus. J. Lab. clin. Med. 69 494 1967
14. Miller M., Craig, J. W. Drucker W. R. & Woodward, H., Jr. The metabolism of fructose in man. Yale J. Biol. Med. 29 335, 1956.
15. Moorhouse, J. A. & Kark, R. M. Fructose and diabetes. Amer. J. Med. 23 46, 1957
16. Noble, R. P. & Campbell, F. M. Improved accuracy in automated fluorometric determination of plasma triglycerides. Clin. Chem. 16 166 1970.
17. Pontremoli, S. & Grazi, E. Gluconeogenesis. In. Carbohydrate metabolism and its disorders. Vol. I (ed. F. Dickens, P. J. Randle & W. J. Whelan), p. 259. Academic Press, London 1968.
18. Rosenkranz, A. & Wagner E. Untersuchungen zur Fruktosetoleranz beim Diabetes mellitus im Kindesalter. Neue öst. Z. Kinderheilk. 6 135 1961
19. Schartow D. Langzeitergebnisse nach Fructoseverabreichung an Diabetiker. Med. Inaugural Dissertation, München 1965. Ref. in Mehnert, H. Die Verwendung von Fructose, Sorbit und Xylit. In Handbuch des Diabetes mellitus. Band II (ed. E. F. Pfeiffer), p. 1069. J. F. Lehmanns Verlag, München 1971.
20. Sols, A. Phosphorylation and glycolysis. In. Carbohydrate metabolism and its disorders. Vol. I (ed. F. Dickens, P. J. Randle and W. J. Whelan) p. 53. Academic Press, London 1968.
21. Wray H. L. & Winegrad, A. I. Free fructose in human cerebrospinal fluid. Diabetologia 2 82, 1966

FRUCTOSE AND LIPID METABOLISM

Acta med. scand. Suppl. 542, pp. 205—214

THE EFFECT OF FRUCTOSE ON HEPATIC SYNTHESIS OF FATTY ACIDS

David Zakim

From the Division of Molecula Biology San Francisco Veterans Administration Hospital and the Department of Medicine U versity f California Medical Center San Francisco California, U.S.A.

Abstract The synthesis of fatty acids from fructose and glucose has been compared in rat and human liver In both species fructose is a better precursor of fatty acids than glucose, primarily as result of the greater rate of glycolysis f fructose as compared to glucose. The high rat of fructose utilization and relatively low rates f glucose utilization by liver is reflected by the activity of fructokinase which is considerably greater than the total hexokinase and glucokinase activities. Although it is not possible to exclude that the high rate f fructolysis produces meta bolic intermediate which alters the functional stat of the fatty acid synthesizing enzymes, the ex perimental evidence indicates that hepatic fatty acid synthesis in carbohydrate-fed animals is limited by the supply of substrate. Because the hepatic metabolism of fructose can supply acetyl CoA to the fatty acid synthesizing enzymes t greater rates than can glucose, hepatic fatty acid synthesis is enhanced in fructose- or sucrose-fed animals. Since estimates f maximum rates of fatty acid synthesis in human liver based on in vitro measurement of the activity f acetyl-CoA carboxylase suggest that d novo synthesis of fatty acids in human liver can be large in comparison to the turnover of triglycerides in very low density lipoproteins, the data are compatible with the conclusion that enhanced rates of hepatic fatty acid synthesis contribute significantly to fructose- or sucrose-induced hypertriglyceridemia in normal men

The observation that substitution of isocaloric amounts of sucrose r fructose for glucose in the diet increases the concentration of plasma triglycerides in man and experimental ani mals (2, 3 7 10—13) has stimulated wide-spread interest in the possible relationships between the source and amount of sugar in the diet and the genesis of premature vascular disease (5 22). Our own interest in this problem has focused on the possibility that the principal mechanism for the selective effects of different sugars on the concentration of plasma triglycerides is via altering the rate of hepatic fatty acid synthesis (7 19, 23—29). Since sucrose is hydrolyzed to glucose and fructose during intestinal absorption, and since fructose when fed to rats as the sole source of dietary carbohydrate has an identical effect on plasma triglyceride con-

centrations as sucrose feeding in man, it appeared reasonable that the mechanisms of fructose and sucrose-induced hypertriglycer idemia in normal animals would be similar In this paper we review the evidence relating to the mechanisms by which fructose as compared to glucose increases the rate of fatty acid synthesis in both rat and human liver The significance of this effect of fructose on hepatic fatty acid synthesis for the produc tion of sucrose-induced hypertriglyceridemia is discussed briefly

MATERIALS AND METHODS

Male rats, 250—350 g, were used in all experiments. Enzymes, substrates, and cofactors were purchased from Sigma Chemical Company Calbiochem, or Boehringer Mannheim. Radioisotopically labelled compounds were purchased from Nuclear Chicago and used without further purifica tion. The techniques used for measurements of glycolytic and Krebs' cycle intermediates, enzyme assays and incorporation of ^{14}C-precursors into fatty acids in liver slices have been described eviously (23—29) The composition of diets used ng nutritional studies and the details of the rimental protocols have also been published 6) Samples of human liver were obtained dur ing lective surgical procedures from patients with chronic cholecystitis or peptic ulcer disease. In no instance were patients j undiced, febrile, or fasted prior to surgery B opsies were taken only from livers which appeared grossly normal t the time of operation.

RESULTS AND DISCUSSION

Substrate flux through a metabolic pathway can be regulated in three basically different ways by the amount of enzyme protein within the pathway by dynamic alteration of the functional state of the enzymes within the pathway and by the availability of substrate. Each of these possible regulatory mechanisms could contribute to the control of hepatic fatty acid synthesis from carbohydrate precursors. Thus, each has been investigated in order to determine its possible importance

Fig 1 Pathway for the synthesis of fatty acids from pyruvate. Abbreviations Ac-CoA, acetyl coenzyme A OAA, oxaloacetate -GP α-glycerophosphate.

for the observed differential effects of glucose and fructose on the concentrations of plasma triglycerides in men and experimental animals. The major pathway for the *de novo* synthesis of fatty acids from carbohydrates is presented diagramatically in Fig 1

The effects of fructose and glucose on the activities of fatty acid synthesizing enzymes in rat liver

That the activities of a variety of enzymes can be inductively increased by the administration of substrates metabolized by these enzymes is well established, and an inductive effect has been demonstrated to take place for fatty acid synthesizing enzymes. Thus, fasting reduces the activities of these enzymes in liver and other tissue whereas feeding a carbohydrate containing diet returns their levels of activity to normal (6). In some instances it is possible to increase the activities of fatty acid synthesizing enzymes to greater than normal levels by suitable modifications of the diet (1). This seems to be the case for fructose feeding

As seen in Table I, feeding rats for 72 hours on a diet containing 69 per cent of calories as fructose increases the hepatic activities of acetyl-CoA carboxylase and

Table I. *Hepatic activities of acetyl-CoA carboxylase and fatty acid synthesis in fructose and chow fed rats. Activities are expressed as mμmoles substrate metabolized per min per mg protein. Data from Zakim and Ho (27)*

Diet	Acetyl-CoA Carboxylase	Fatty Acid Synthetase
Chow	2.29	2.96
Fructose	4.87	10.86

Table II. *Comparison of the effect of fructose and glucose feeding on the activities of hepatic fatty acid synthesizing enzymes. Activities are expressed as mμmoles substrate metabolized per min per mg protein. Data from Zakim et al. (28)*

Diet	NADP Malate Dehydrogenase	Citrate Cleavage	AcCoA Carboxylase
Purina Chow	8.72	3.55	5.19
Glucose	23.3	10.40	8.75
Fructose	23.1	10.73	9.60

fatty acid synthetase to levels greater than those seen in animals fed a standard chow diet. Thus, one of the ways in which fructose feeding can enhance the hepatic synthesis of fatty acids is by increasing the amounts of fatty acid synthesizing enzymes. It is known, however that glucose feeding may have a qualitatively similar effect on fatty acid synthesizing enzymes. For this reason a direct comparison of the effects of feeding large amounts of glucose or fructose on the activities of fatty acid synthesizing enzymes was carried out. For these experiments rats were fed diets containing 80 per cent of calories as fructose or glucose for two days. At the end of this period animals were killed and the activities of three fatty acid synthesizing enzymes measured. As seen in Table II fructose and glucose feeding had quantitatively identical actions on the activities of acetyl-CoA carboxylase citrate cleavage enzyme, and NADP malate dehydrogenase increasing each by 2 to 3-fold. Although the activity of fatty acid synthetase was not measured in this study the activity of acetyl CoA carboxylase is rate limiting for the synthesis of fatty acids in the presence of saturating amounts of substrates (8, 14, 15). These data indicate, therefore, that although fructose-induced increases in the activities of fatty acid synthesizing enzymes in rat liver can contribute to fructose-induced hypertriglyceridemia, they cannot account for the differential effects of this sugar as compared to glucose. It should be pointed out, however that since large amounts of glucose increase the maximum capacity for hepatic fatty acid synthesis, variations in the glucose content of the diet may also be associated with changes in the concentration of plasma triglycerides in normal animals.

The conversion of ^{14}C fructose and ^{14}C-glucose to fatty acids in liver slices

Intravenous administration of fructose as compared to glucose is associated with a large increase in the concentration of plasma pyruvate (12). Studies with liver slices also document that fructose is metabolized more rapidly than glucose, and that these differences cannot be accounted for by substrate induction of specific fructose metabolizing enzymes (16). It appeared important, therefore to investigation the conversion of fructose and glucose to hepatic fatty acids using experimental conditions which did not allow for alteration of the amount of enzymes along the pathway from fructose or glucose to fatty acids. For these studies the conversion of 5.0 mM ^{14}C fructose or 5.0 mM ^{14}C-glucose to fatty acids was measured in liver slices incubated in Krebs-Henseleit solution (28). The production of $^{14}CO_2$ was also monitored. In animals fed a normal diet of Purina rat chow liver slices converted ap-

Table III. *Comparison of the conversion of ^{14}C fructose and ^{14}C-glucose to fatty acids and CO_2 in liver slices from rats fed a chow diet. Values given for products formed are the mean ± SE of mμmoles ^{14}C fructose or ^{14}C-glucose recovered per 100 mg tissue per 3 hour incubation. Tissue from 8 different rats was used for each experiment. Data from Zakim et al. (28)*

Product	Labelled Substrate ^{14}C Fructose	Labelled Substrate ^{14}C-Glucose
Fatty Acids	16.8 ± 6.1	3.7 ± 1.5
CO_2	602.4 ± 93.4	170.3 ± 20.9

Table IV. *Comparison of the conversion of 5.0 mM ^{14}C fructose and ^{14}C-glucose to fatty acids, glyceride glycerol, CO_2 and glycogen in human liver slices. Values given for products formed are mμmoles ^{14}C fructose or ^{14}C-glucose recovered in each product per 100 mg tissue per 90 min incubation and are the means of duplicate incubations. All data in this Table are from the same liver and are taken from Zakim et al. (26)*

Product	Labelled Substrate Fructose	Labelled Substrate Glucose
Fatty Acids	43.0	5.5
Glyceride-Glycerol	56.3	15.8
CO_2	337.0	70.4
Glycogen	63.3	5.1

proximately four times more ^{14}C-fructose than ^{14}C-glucose to fatty acids (Table III) indicating that fructose was a superior precursor of fatty acids than was glucose, in experiments in which there was no contribution to fatty acid synthesis from inductive effects on the amounts of fatty acid synthesizing enzymes. A similar result was obtained in studies with human liver slices. The data in Table IV ... data from a typical experiment ... that in the human liver fructose is ... better precursor of fatty acids than is ...cose. Also, more ^{14}C fructose than ^{14}C-glucose was converted to CO_2, glycogen, and glyceride-glycerol in these slices.

The above experiments indicated that differences in the dynamic regulation of fructose and glucose metabolism were responsible for the varying effect of these sugars on hepatic fatty acid synthesis. However in view of the data in Tables I and II which suggest that diet can influence the rate of hepatic fatty acid synthesis by inductive effects on the amounts of fatty acid synthesizing enzymes, the influence of prior feeding of 80 per cent of calories as fructose or glucose on the subsequent conversion of ^{14}C-sugars to fatty acids was also studied in rat liver slices. For obvious reasons, it was not possible to do similar experiments with human liver. As expected from the data in Tables I and II feeding the high glucose or fructose diets increased the conversion of both ^{14}C fructose or ^{14}C-glucose to fatty acids. (Tables III and V). However irrespective of the antecedent diet, more ^{14}C-fructose than ^{14}C-glucose was always recovered as fatty acids. On the other hand, the difference between the amounts of ^{14}C fructose and ^{14}C-glucose recovered as fatty acids was influenced by the dietary history of the animals in that the difference was greatest (7 fold) in livers from fructose fed rats and least (2 fold) in livers from glucose fed animals (Table V). Thus, although glucose and fructose feeding increase the activities of fatty acid synthesizing enzymes to the same extent, each sugar had a selective enhancing effect on its own conversion to fatty acids in rat liver slices. This was a time dependent effect, suggesting that the selective enhancement of the fructose or glucose diet on the synthesis of fatty acids from fructose or glucose respectively was due to inductive changes in portions of the metabolic pathway proximal to the fatty acid synthesizing enzymes. The conclusion that the differential synthesis of fatty acids from fructose or glucose is based on events prior to the entry of substrate into the fatty acid synthesizing pathway was strengthened by the observation that no matter which products

Table V *Effect of diet on the conver[illegible] ^{14}C fructose and ^{14}C-glucose to fatt[illegible] and CO_2 in rat liver slices. Values gi[illegible] products formed are the mean ± SE [illegible] moles ^{14}C substrate recovered as [illegible] per 100 mg tissue per 3 hours. Th[illegible] 8 different animals was used for e[illegible] iment. Data from Zakim et al. (8*

Diet	Product	Labelled S[illegible] Fructose
Fructose	Fatty Acid	74 2 ± 12 1
	CO_2	709 4 ± 83 6
Glucose	Fatty Acid	46 0 ± 11 0
	CO_2	602.4 ± 93 4

were measured more ^{14}C from f uctose was recovered than ^{14}C from glucose. Also, the data for glycogen (Table IV) ex lud the possibility that isotope dilution effects can account for the observed differences in the recoveries of ^{14}C-glucose and ^{14}C fructose in the other products examined.

Enzymatic capacity for the glycolysis of fructose and glucose in liver cells

Examination of the activities of enzymes concerned with the glycolysis of fructose and glucose in the liver yields some clues as to the reason why glucose is a less useful precursor for fatty acid synthesis than fructose. Thus, as shown in Table VI, the rate at which fructose can be phosphorylated in liver cells from adult rats is potentially several fold greater than that for glucose since fructokinase activity is high in comparison to the activity for glucokinase and hexokinase. It should be mentioned that although the relative levels of glucokinase and fructokinase can be altered by dietary manipulations, fructokinase activity always exceeds the combined capacity for the synthesis of glucose-6-P by glucokinase and hexokinase. For example, glucose feeding

Table VI. *Activities for phosphorylating enzymes for fructose and glucose in rat liver. Activities are mμmoles substrate metabolized per min per mg protein. Data from Stifel et al. (19)*

Diet	Fructo-kinase	Gluco-kinase	Hexo-kinase
ructose	43 8	6.65	2.4
lucose	23.1	14 0	6.3

increases the glucose-phosphorylating capac ty of the liver (Table VI) which probably accounts for the observation that the glucose diet diminishes the extent of the excess fatty acid synthesis from fructose as compared to glucose (Table V) but more ^{14}C-fructose than ^{14}C-glucose is recovered as fatty acids even in these animals. A similar situation exists in the human (26).

Fig 2 shows schematically the general pathways and enzymes involved in the glycolysis of glucose and fructose and the data in Table VII give a more complete profile of the enzymatic capacity for glycolysis in human liver. Especially important in this Table is the demonstration that despite its high activity the activities of glycolytic enzymes downstream from the fructokinase

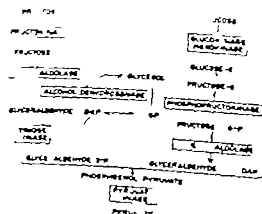


Fig. 2 Glycolytic pathways for glucose and fructose. Abbreviations DAP dihydroxyacetone-P α-GP α-glycerophosphate.

Table VII. Comparison of enzyme activities for the glycolysis of fructose and glucose in human liver. Activities are the mean ± S.E. of mμmoles substrate metabolized per min per mg protein. The figure in parenthesis is the number of samples from different livers. Data from Zakim et al. (26)

Fructose enzymes	Activity		Glucose enzymes	Activity	
Fructokinase	77.4 ± 3.6	(7)	Glucokinase	3.13 ± 0.31	(6)
F 1 P Aldolase	50.4 ± 2.8	(4)	Hexokinase	1.76 ± 0.25	(6)
Triokinase	34.6	(2)	Phosphofructokinase	30.5 ± 5.5	(7)
Alcohol dehydrogenase			F 1 6-P Aldolase	54.7 ± 7.0	(4)
NADH	65.3 ± 5.7	(7)	Pyruvate Kinase	221 ± 29.4	(3)
NADPH	[illegible]8.2 ± 6	(6)			

reaction are at least as great as the capacity of the liver for phosphorylation of fructose. On the other hand, at saturating concentrations of glucose, the synthesis of glucose-6-P is still likely to be rate limiting for the overall glycolysis of glucose in the liver cell. Although it has recently been shown by Woods, Eggleston and Krebs (21) that fructose-1-P aldolase is inhibited competitively by IMP during fructose metabolism, thereby [illegible]ng to large [illegible]creases in the hepatic con[illegible] of [illegible]ose-1 P it is still unlikely [illegible] the rate [illegible] [illegible]ysis of fructose falls [illegible]elow that fo[illegible] [illegible]ose.

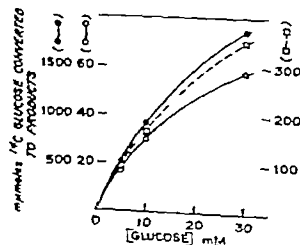


Fig. 3. Dependence of fatty acid synthesis by human liver slices on glucose concentration. Data is mμmoles of ^{14}C-glucose recovered as fatty acids (O), CO_2 (●), and glyceride-glycerol (□)

In addition to the low maximum activity for the phosphorylation of glucose, the hepatic enzyme primarily responsible for this phosphorylation, glucokinase, has a poor affinity for glucose, the K_m for glucose being approximately 10 mM (9). Thus, even during the active absorbtion of sugar glucokinase is likely to be less than one half saturated with substrate (blood sugar concentration of less than 180 mg per 100 ml). It follows that if in some way the slow rate of glycolysis of glucose is responsible for its relatively poor conversion to fatty acids in comparison to fructose, the conversion of glucose to fatty acids should be highly concentration dependent within the physiologic range of glucose concentrations. This in fact is the case as shown for human liver slices in Fig. 3. Increasing the medium concentration of ^{14}C-glucose from 5.0 mM (90 mg per 100 ml) to 30 mM increases the rate of conversion of ^{14}C-glucose to fatty acids, and, in addition, to glyceride-glycerol and CO_2. It seems clear that the greater hepatic production of fatty acids from fructose than glucose is in some way dependent on the greater rate of glycolysis of the former sugar in comparison to the latter. Two possible mechanisms could contribute to this effect. Either a glycolytic or Krebs' cycle intermediate is important for the dynamic regulation of fatty acid synthesis or the synthesis of fatty acids is limited by the availability of substrates.

Effect of fructose on hepatic concentrations of glycolytic and Krebs cycle intermediates

As mentioned above, it is well known t the administration of fructose increases serum concentration of pyruvate and . Studies in animals have confirmed that a u administration of fructose increases the te J state level of several glycolytic and Kre cycle intermediates in liver (4, 21) Chron c administration of fructose, as for example by *ad libitum* feeding of diets containing larg amounts of fructose is also associated with increased hepatic concentrations of several metabolic intermediates along the pathway from fructose to fatty acids, including acetyl CoA (28). Two studies in intact rats have demonstrated that the concentrations of some intermediates may increase only transiently despite the continued availability of relatively large concentrations of fructose in the blood (8, 23). Because, however of considerable uncertainty as to the exact mechanism for the dynamic regulation of the fatty acid synthesizing pathway more specifically the activity of acetyl-CoA carboxylase, the signif icance of the effects of fructose on the concentrations of metabolic intermediates is dif ficult to determine. It is important, never theless, to consider two intermediates, α-glycerophosphate and citrate, which have been postulated to be of importance for the regula tion of fatty acid synthesis.

It has been proposed that fatty acid synthesis can be controlled by its production of cy- CoA compounds, which inhibit acetyl-CoA carboxylase *in vitro* (see ref. 25 for a brief review of this problem). Since acyl-CoA derivatives react with α-glycerophosphate the concentration of this latter compound could modulate theoretically the rate of fatty acid synthesis by increasing or decreasing the removal of acyl-CoA. More recent information indicates that it is unlikely that acyl-CoA compounds are important physiogically for the regulation of acetyl-CoA arboxylase (25) On the other hand, modulati of the rate of esterification of acyl-CoA variations in the concentration of α-glycep osphate could alter the ratio of bound free CoA which is known to influence the rate of oxidation of pyruvate to acetyl CoA. Since this is an intermediate step for the conversion of carbohydrate carbon to fatty acids, inhibition of pyruvate dehydrogenase might limit the synthesis of fatty acids. How ever there is no correlation between *in vivo* rates of esterification of fatty acids and the hepatic concentration of α-glycerophosphate (25) so the significance of the concentra tion of α-glycerophosphate for metabolic regulation is uncertain. In the absence of knowledge of the factors controlling the rate of esterification, or for that matter the details of the pathway from fatty acid synthetase bound acyl chain to complex lipids, it is impossible to state whether or not esterification can directly influence the rates of fatty acid synthesis in livers from fed animals. On the other hand, it is worth pointing out, as the data in Tables I to IV show that the amount of carbon metabolized to CO_2 or glyceride-glycerol is far in excess of that recovered as fatty acids with either glucose or fructose as substitute. Furthermore, since 4 moles of glucose are required for the synthesis of 1 mole of palmitate, there is sufficient carbon in 1 mole of glucose to potentially esterify the palmitate derived from 24 moles. In view of these considerations and the data cited above (Tables I to IV) it seems unlikely that the flux of carbon from glucose to α-glycerophosphate could limit the incorporation of glucose into fatty acids.

Citrate, the other metabolic intermediate which could effect the functional state of acetyl-CoA carboxylase *in vivo* is required *in vitro* for assay of acetyl-CoA carboxylase. Citrate acts by converting an inactive monomeric form of the enzyme to an enzymatically

Table VIII. *Comparison of tissue substrate concentrations to K_m values for the conversion of citrate to malonyl-CoA*

	Reaction	
	Citrate → Acetyl-CoA	Acetyl CoA → Malonyl-CoA
K_m for substrate	5.8×10^{-4}M	1.9×10^{-5}M
Tissue substrate concentration	4×10^{-4}M	4.3×10^{-5}M

active polymeric form. As with α-glycerophosphate, however the importance of this *in vitro* citrate requirement is uncertain in that the concentrations of citrate required for in *vitro* activation are far larger than *in vivo* citrate concentrations (25) Also it has recently been shown that acetyl-CoA carboxylase can be isolated directly in an active form from livers of fed animals (20)

On the basis of the above arguments and data α-glycerophosphate or citrate are not [illegible] primary factors for altering the [illegible] fatty acid synthesis *in vivo* [illegible] able to conclude therefore, [illegible] or reason for the enhanced con[illegible]n of fructose to fatty acids as compared [illegible] glucose is that the rate of hepatic fatty acid synthesis is limited by the availability of substrate in fed animals. Since fructose is metabolized to pyruvate and acetyl-CoA at greater rate than glucose fructose feeding increases hepatic fatty acid synthesis to a greater extent than glucose. Consideration of the relationships between the K_ms of some fatty acid synthesizing enzymes and the *in vivo* concentrations of their substrates is compatible with this idea. Thus, the tissue concentrations of citrate and acetyl-CoA are close to the K_m for citrate cleavage enzyme and K_m for acetyl-CoA carboxylase (Table VIII) Since these substrates are not evenly distributed within the cell, their concentrations in the cytosol, the primary site of fatty acid synthesis, could be far less than their K_ms and increases in the concentration of these substrates could effect large increases in the rate of fatty acid synthesis. The total magnitude of this effect is likely to be larger than one would expect from an inspection of Table VIII for as shown by Srere (18) the concentrations of enzymes in liver cells are large in comparison to the concentrations of their substrates.

Table IX. *Acetyl-CoA co boxylase activity in human liver Activity is expressed as mμmoles $H^{14}CO_3$ fixed per min per mg protein*

Patient	Acetyl-CoA Carboxylase Activity
1	08
2	3.98
3	0.438
4	1.59
Mean	2.0[illegible]

Quantitative importance of hepatic fatty acid synthesis in man. Unfortunately there is no direct measure of the absolute rate of hepatic fatty acid synthesis in man or any other large mammal, and in order for the enhanced conversion of fructose to fatty acids in liver to be of physiologic importance it would have to be shown that human liver can synthesize fatty acids at a rate consistent with the magnitude of dietary induced changes in in the concentration of plasma triglycerides. In an attempt to obtain an approximate value for maximal rates of hepatic fatty acid synthesis we measured acetyl-CoA carboxylase activity in samples of human liver obtained during elective abdominal surgery The data in each of 4 patients are listed in Table IX. It may be of importance that although patient No. 3 appeared to be eating normally prior to surgery he had a partial gastric obstruction secondary to a duodenal ulcer and the low hepatic acetyl-CoA carboxylase acti[illegible] in this patient as compared

have been related to a subtle nutritional deficiency

Based on an average acetyl-CoA carboxylase activity of 2.02 m μmoles $H^{14}CO_3$ fixed/min/mg protein and a liver weight of 3 per cent body weight, one can calculate that the liver in a 70 kg man could synthesize maximally 1 g of triglyceride/hr With an acetyl-CoA carboxylase activity of 3.98 mμmoles $H^{14}CO_3$ fixed/min/mg this value could be as high as 2 g/hr Since the turnover rate of very low density plasma triglycerides in men with normal plasma triglyceride concentrations is from 2.0 to 10.7 mg/kg/hr (17) the rate of *de novo* triglyceride synthesis in the liver could be large in comparison to the turnover rate of the plasma very low density lipoprotein triglyceride. Although it is not possible to state with certainty on the basis of the acetyl-CoA carboxylase activities alone what the actual rates of hepatic fatty acid synthesis are, it is reasonable to conclude that *de novo* synthesis of fatty acids in the human liver is a quantitatively significant factor in the turnover of plasma triglyceride fatty acids. Although an effect on hepatic fatty acid synthesis is only one of several possible ways in which fructose containing sugars could increase the plasma triglyceride concentration, the observed effects of different carbohydrate diets on the plasma lipid concentrations in man and animals is consistent with predictions derived from the data on the more rapid *de novo* conversion of fructose to hepatic fatty acids as compared to glucose.

REFERENCES

1 Allman, D. W Hubbard, D & Gibson, D M F tty acid synthesis during fat free refeeding of starved rats. J Lipid Res. 6 63, 1965

2 Anderson, J T Grande, F Matsumoto, Y & Keys, A Glucose, sucrose and lactose in the diet and blood lipids in man. J Nutr 79 349 1963.

3 Antonis, A. & Bersohn, I. The influence of diet on serum triglycerides in South African white and Bantu prisoners. Lancet 1 3, 1961

4 Burch, H. B., Lowry O H Meinhardt, L., Max P & Chyn, K. Effect of fructose, dihydroxyacetone, glycerol, and glucose on metabolites and rel ted compounds in liver and kidney J biol. Chem 245 2092, 1970

5 Cohen, A. M., Barby S. & Poznanski, R. Change of diet of Yemenite Jews in relation to diabetes and ischaemic heart disease Lancet ii 1399, 1961

6 Ga guly J Studies on the mechanism of fatty acid synthesis VII. Biosynthesis of fatty cids from malonyl CoA Biochim biophys. Acta 40 110 1960

7 Herman, R. H Zakim, D & Stifel, F B. Effect of diet on lipid metabolism in experimental animals and man. Fed. Proc. 29 1302, 1970.

8 Kupke, I. & Lamprecht, W Über di Biosynthese von Lipiden aus Fructose in Leber I. Einbau von uniform markieter [^{14}C] Fructose in Leberlipide. Z. physiol Chem. 348 17 1967

9 Lea, M. A. & Walker D G Hepatic glycolysis and some changes that occur during development. Biochem. J 94 655 1965.

10 Macdonald, I. & Braithwaite, D M. The influence of dietary carbohydrates on the lipid pattern in serum and in adipose tissue. Clin Sci. 27 23, 1964.

11 Macdonald I. The effects of various dietary carbohydrates on the serum lipid levels during five day regimen Clin. Sci. 29 193, 1965.

12 Miller M. Drucker W R., Owens, J E., Craig, J W & Woodward H Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J Clin. Invest. 31 115, 1952.

13 Nikkilä, E. A. & Ojala, K. Induction of hyperglyceridemia by fructose in rat Life Sci 4 937 1965.

14 Numa, S., Matsuhashi, M. & Lynen, F Zur störung der Fettsäuresynthese bei Hunger und Alloxan-diabetes. I. Fettsäuresynthese in der Leber normaler und hungernder Ratten Biochem. Z 334 203, 1961

15 Numa, S., Bortz, W M. & Lynen, F Regulation of fatty acid synthesis t the acetyl CoA carboxylation step. Advan. Enzyme Regulat. 3 407 1965.

16 Renold, A. E., Hastings, A. B. & N F B. Studies of carbohydrate meta rat liver slices. III. Utilizat and fructose by liver from n animals. J biol. Chem. 2

17 Reaven, G M., Hill, D B., Gross, R. C. & Farquhar J V., Kinetics of triglyceride turnover of very low density lipoproteins of human plasma. J clin. Invest. 44 1826, 1965.

18. Srere, P., Enzyme concentrations in tissues. Science 158 936, 1967

19 Stifel, F B., Rosensweig, N. S., Zakim, D & Herman, R. H., Dietary regulation of glycolytic enzymes. I. Adaptive changes in rat jejunum. Biochim. biophys. Acta 120 221, 1968.

20. Welbourne, W P Swanson, R. F & Suber H. S., Active acetyl CoA carboxylase from liver of rats fed carbohydrate. Biochem. biophys. Res. Comm. 37 933, 1969.

21. Woods, H. F., Eggleston, L. V & Krebs, H. A., The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. Biochem. J 119 501, 1970.

22. Yudkin, J., Evolutionary and historical changes in dietary carbohydrates. Amer J clin. Nutr. 20 108, 1967

23. Zakim, D. & Herman, R. H., The effect of intravenous fructose and glucose on the hepatic α-glycerophosphate concentration in the rat. Biochim. biophys. Acta 165 374, 1968.

24. Zakim, D. & Herman, R. H., The effect of clofibrate on the serum triglyceride concentration in normal males fed high-sucrose diets. J Atheroscler Res. 10 91, 1969.

25. Zakim, D. & Herman, R. H. Regulation of fatty acid synthesis. Amer. J clin. Nutr 22. 200 1969

26. Zakim, D. & Ho, W., The acetyl CoA carboxylase and fatty acid synthetase activities of rat intestinal mucosa. Biochim. biophys. Acta 222 558, 1970.

27 Zakim, D Herman, R. H. & Gordon, W C., The conversion of glucose and fructose to fatty acids in the human liver Biochem. Med. 2. 427 1969

28. Zakim, D Pardini, R. S., Herman, R. H. & Sauberlich, H. E. Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver Biochim. biophys. Acta 144 242, 1967

29 Zakim, D Pardini, R. S Herman, R. H. & Sauberlich, H. E. The relation of hepatic α-glycerophosphate concentration to lipogenesis in rat liver Biochim. biophys. Acta 137 179, 1967

EFFECT ON SERUM LIPIDS OF DIETARY SUCROSE AND FRUCTOSE

Ian Macdonald

From the Department of Physiology Medical School, G y' Hospital, London S.E.1 9 RT U.K.

Abstract. Though the measurement of the concentration f substances in the serum may provide misleading evidence for metabolic studies, never theless findings of changes in the levels of various serum lipids have a high degree of association with vascular disease. Sucrose or one of its constituent monosaccharides, fructose, has been found to have an influence on serum triglyceride concentration — the effect on the serum cholesterol level being minimal — when compared with glucose or its polymers.

After the acute ingestion of sucrose less striking fall is found in the serum triglyceride level than after glucose ingestion. Experimental diets containing a large proportion of carbohydrate result in level of triglyceride that is higher when the carbohydrate contained fructose than when it contained glucose. The effect of dietary fructose on the fasting level of serum triglyceride is modified by the sex f the consumer the amount and type of fat accompanying the fructose, the type of protein in the diet, the frequency of intake, etc.

The few studies on long term ingestion of fructose suggest that the rise in triglyceride concentration following fructose ingestion falls.

There is some evidence that in vascular disease the fructose metabolism may be more disturbed than that of glucose.

The concentrations of various substances in the blood have long been used as indicators of metabolic disarray or impending disease. As blood is relatively plentiful and easy to obtain it is not surprising that exhaustive studies have been made of its composition, especially in disorders that are common and for which prediction and consequent preven tion are still largely unknown. In arterial disease the lesion in the wall is fatty and in the absence of an easy method to assess the cause and extent of this disease the lipid components of the blood have been examined. A close association has been found between the complications of arterial disease and both the serum cholesterol and triglyceride levels, but no direct relationship has been shown, and in fact it is not unknown for the coronary arteries to become blocked as a consequence of arterial disease and the serum levels of both cholesterol and tri glyceride to be within normal limits at the time. Consequently the effect of diet on serum lipids may be of purely academic in terest or it may have considerable clinical importance It is not the level of lipid in the serum per se that matters it is the disability that may arise or be associated with this elevation in concentration that is important.

DIETARY SUCROSE/FRUCTOSE IN HYPERLIPIDAEMIA

As was pointed out in 1961 (1) and subsequently confirmed in a classification of hyperlipidaemia (13) there are some patients who are carbohydrate-sensitive in that the fasting serum triglyceride concentration increases as the intake of carbohydrate increases. The incidence of this condition is not known, nor is it known whether the triglyceride level of these patients is more sensitive to sucrose/fructose than to glucose and its polymers.

Vascular disease is a complication associated with carbohydrate-induced hypertriglyceridaemia (13) and it has been found that patients with peripheral vascular disease have higher levels of serum fructose after ingesting sucrose than do controls (30). Several weeks after a myocardial infarct the amount of ^{14}C, from both glucose and fructose, incorporated into the serum triglycerides is much greater than in the controls (24). More the radiolabel from fructose was incor -d into the serum triglycerides than glucose. This was found to apply to both the glycerol and fatty acid moieties of the serum triglyceride (33).

CONCLUSIONS

Thus it seems clear that the fructose component of the diet can give rise to responses of lipid metabolism that are different from those found after consuming glucose. The response of the serum triglyceride concentration to dietary glucose seems to be unaffected by those many factors that affect the serum triglyceride response to fructose. Included in the factors that modify the dietary fructose/serum triglyceride relationship are the sex of the consumer the type of dietary fat accompanying the fructose, the frequency of eating the fructose, and the source of amino nitrogen in the diet.

REFERENCES

1. Ahrens, E. H., Hirsch, S., Oette, K., Farquhar J W & Stein, Y.. Carbohydrate-induced and fat-induced lipemia. Trans. Ass. amer. Physicians 74 134, 1961
2. Albrink, M. J Fitzgerald, J R. & Man, E. B.. Reduction of alimentary lipaemia by glucose. Metabolism 7 162, 1958.
3. Antar, M. A. & Ohlson, M. A.. Effect of simple and complex carbohydrates upon total lipids, non-phospholipids and different fractions of phospholipids in young men and women. J Nutr 85 329, 1965.
4. Antar M. A., Little, J A., Lucas, C., Buckley G C. & Csima, A.. Synergistic effect of sucrose and animal fat on serum lipids. Atherosclerosis 11 191, 1970.
5. Antonis, A. & Bersohn, I.. The influence of diet on serum triglycerides in South African white and Bantu prisoners. Lancet 1 3, 1961.
6. Barter P J Carroll, K. F & Nestel, P J Diurnal fluctuations in triglyceride free fatty acid, and insulin during sucrose consumption and insulin infusion in man. J clin. Invest. 50 583, 1971.
7. Beveridge, J M. R., Jagannathan, S. N. & Connell, W F.. The effect of the type and the amount of dietary fat on the level of plasma triglycerides in human subjects in the post-absorptive state. Canad. J Biochem. 42. 999 1964.
8. Bragdon, J H., Havel, R. J & Gordon, R. S.. Effects of carbohydrate feeding on serum lipids and lipoproteins in the rat. Amer J Physiol. 189 63, 1957
9. Coltart, T M. & Macdonald, I. Effect of sex hormones on fasting serum triglycerides in baboons given high sucrose diets. Brit. J. Nutr 25 323, 1971
10. Crossley J N.. Sucrose tolerance and fasting serum triglyceride concentrations in young men and women. Proc. Nutr Soc. 26 iii, 1967.
11. Elias, R. & Macdonald, I.. To be published 1972.
12. Fabry P.. Feeding patterns and nutritional adaptation. Butterworth, London, 1967
13. Fredrickson, D S., Levy R. I. & Lees, R. S.. Fat transport in lipoproteins — an integrated approach to mechanisms and disorders. New Engl. J Med. 276 34, 94, 148, 215, 273, 1967.
14. Grande, F Dietary carbohydrates and serum cholesterol. Amer J clin. Nutr 20 1 6, 1967
15. Havel, R. J Early effects of fasting and of carbohydrate ingestion on lipids and lipopro-

teins of serum in man. J clin. Invest. 36 855, 1957

16. Jones, D. S. & Arky R. A.. Effects of insulin on triglyceride and free fatty acid metabolism in man. Metab. clin. exp. 4 1287 1965

17 Jourdan, M. H. A difference between male and female baboons in the serum glyceride response to rapid intravenous injections of fructose. J Physiol. (Lond). 201 27 1969

18. Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y Effect of fructose, glucose, sucrose and starch on serum lipids in car bohydrate-induced hypertriglyceridaemia and in normal subjects. Israel. J med. Sci. 2 715 1966.

19 Klugh, C. A. & Irwin, M. L Serum lipid levels of young women as related to source of dietary carbohydrate. Fed. Proc. 25 672, 1966.

20. Kuo, P T & Bassett, D R. Dietary sugar in the production of hyperglyceridaemia. Amer J int. Med. 62 1199 1965.

21 Macdonald, I. The lipid response f young women to dietary carbohydrates. Amer J clin. Nutr 16 458, 1965.

22. Macdonald, I. Influence of fructose and glucose on serum lipid levels in men and pre- and post menopausal women. Amer. J clin. Nutr 18 369 1966

23. Macdonald, I. Inter relationship between the influences of dietary carbohydrates and fats on fasting serum lipids. Amer J clin. Nutr 200 345, 1967

24. Macdonald, I. Ingested glucose and fructose in serum lipids in healthy men and after myocardial infarction. Amer J clin. Nutr 21 1366, 1968.

25. Macdonald, I. Effects f dietary glycerol on the serum glyceride level of men and women. Brit. J Nutr 24 537 1970.

26. Macdonald, I. Inter relationship f dietary carbohydrates and fats on serum lipid concentrations. Proc. Nutr Soc. 30 72 A, 1971

27 Macdonald, I Effects of an amino acid/ca bohydrate diet on fasting serum lipid levels In Balanced Nutrition and Therapy,p 100 Ed. Lang, K., Fekl, W & Berg, G Verlag, Stutt gart, 1971

28. Macdonald, I & Braithwaite, D. M. The influence of dietary carbohydrates on the lipid pattern in serum and in dipose tissue Clin. Sci. 27 23, 1964.

29 Macdonald, I Coles, B. L., Brice, J & Jourdan, M. H. The influence of frequency of sucrose intak on serum lipid, protein and carbohydrate levels. Brit. J Nutr 24 413, 1970

30 Macdonald, I. & Turner L. J Serum glucose and fructose levels after sucrose meals in therosclerosis. Nutr Metab. 13 168, 1971

31. Mann, J I., Truswell, A. S. & Pimstone, B. L. The different effects of oral sucrose and glucose on alimentary lipaemia. Clin. Sci. 41 123, 1971

32. Marks, V & Samols, S. E. Intestinal factors in the regulation of insulin secretion. I Advances in Metabolic Disorders» Ed. Levine R. & Luft, R. Academic Press. N Y 4 1, 1970

33. Maruhama, Y Conversion of ingested car bohydrate ^{14}C into glycerol and fatty cids f serum triglycerid in patients with myoca dial infarction. Metabolism 19 1085 1970.

34 Meyer F L., Mattox, H., Bolick, M. & Mac donald, C. Metabolic changes after test meals with different carbohydrates blood levels of pyruvic acid, glucose and lactic dehydrogenase. Amer J clin. Nutr 24 615, 1971

35 Perry W F & Corbett, D N Changes in plasma triglyceride concentration following the intravenous administration of glucose. Canad. J Biochem. 42 353, 1964.

36 Reaven, G M Lerner R. I Stern, M. P & Farquhar J W Role of insulin in endogenous hypertriglyceridemia. J clin. Invest. 46 1756, 1967

37 Roberts, A M. Some effects of sucrose free diet on fasting serum lipid levels. Proc. N tr Soc. 30, 71 A, 1971.

38. Sullivan, J F The effect of protein ingestion on alimentary lipemia. Amer J med. Sci. 243 770, 1962.

39 Swan, D C., Davidson, P & Albrink, M. J Effects of simple and complex carbohydrates on plasma non-esterized fatty acids, plasma sugar and plasma insulin during oral carbohydrat tolerance tests. Lancet 1 60, 1966.

Acta med. scand. Suppl. 542, pp. 221—227

EFFECTS OF DIETARY FRUCTOSE AND SUCROSE ON PLASMA TRIGLYCERIDE METABOLISM IN PATIENTS WITH ENDOGENOUS HYPERTRIGLYCERIDEMIA

Esko A. Nikkilä and Matti Kekki

From the Third Department of Medici e, University Central Hospital, Helsinki, Finland

Abstract. Since fructose is now marketed as a nutrient study has been initiated to compare the effects of fructose, sucrose and starch t a conventional dietary intake level on the plasma triglyceride concentration and turnover in patients with endogenous hypertriglyceridemia. Preliminary results are presented here.

Fructose or sucrose in a daily amount f 75 to 80 grams were added to the diet in an isocaloric exchange for starch in twelve hyperglyceridemic patients under hospital conditions. Each dietary period lasted for 10 to 20 days and the triglyceride turnover was studied by endogenous glycerol labeling technique at the end of each period. Five of the patients had manifest diabetes managed by diet or oral antidiabetic drugs

Plasma triglyceride response to fructose was variabl and the verage change from the level observed during fructose-free diet was not sig nificant. However in nondiabetic subjects a definite rising trend of triglyceride was noticeable. Sucrose caused significant increase of triglyceride concentration above that measured during fructose and starch diets. Diabetics were not included into the sucrose group, however The fractional removal rate of plasma triglycerides was identical during all three diets but the triglyceride production rate showed a rising trend during fructose and, particularly during sucrose diet as compared to starch. Because f the limited number of subjects studied so far these results do not justify any definite conclusions.

It is now generally recognized that high carbohydrate intake tends to elevate the plasma triglyceride concentration and among dif ferent carbohydrates sucrose and fructose show the highest hyperglyceridemic activity (for review see (24)) In fact, the effect of sucrose has been attributed to its fructose moiety and the increase of plasma triglyceride concentration is believed to result from a greater incorporation of exogenous fructose than of glucose into hepatic and plasma triglycerides (3 23) The main part of the human dietary studies carried out on influences of either sucrose or fructose on plasma lipids have used the sugars in amounts that far exceed those usually contained in the average diet of adult man (2, 4 11—14 19) In these experiments all dietary carbohydrate has been either starch or simple sugar and this has resulted in the very unusual daily sugar intakes of 200 to 500 grams. In one

study using more physiological amounts of sugars no difference was found in serum lipid levels on exchanging starch and sucrose in normoglyceridemic subjects (9) Comparison between the effects of dietary sucrose and fructose has previously been carried out in only one human experiment (12, 13).

Thus, in spite of a considerable number of dietary studies it is not yet clear whether the sugar intake at an average customary level has any influence on plasma triglyceride concentration in normal subjects or in patients with familial hypertriglyceridemia. The present study was undertaken to determine the plasma triglyceride response of hyperglyceridemic patients to interchanges of dietary sucrose, fructose and starch. Furthermore, the effect of the sugars on triglyceride transport kinetics was assessed in these experiments. This paper records the preliminary data of a more extensive study still in progress.

MATERIAL AND METHODS

Twelve subjects with endogenous type IV hypertriglyceridemia are included in this report. Four were women aged 59 to 62 years and eight were men ranging from ?6 to 67 years. Five patients had manifest diabetes which was either untreated or controlled by oral antidiabetic drugs. This drug treatment was not changed during the dietary experiments. No drugs influencing directly the lipid metabolism were used either before or durin the study The patients had been originally detected because of symptoms of coronary heart disease obesity or diabetes but at the time of the study none had had a recent coronary event.

All subjects were hospitalized and put on an approximately isocaloric mixed hospital diet. The experimental period was started when the plasma triglyceride concentration had stabilized to certain level, which in most instances was much less than the value on dmittance. Thus, the home diet was qualitatively or quantitatively more hyperglyceridemic than the standard hospital regimen. The fall of plasma triglyceride might possibly be ascribed to the bsence of alcohol in the hospital. Many of the patients had certainly consumed it under home conditions even though none was known to be alcoholic.

The actual experiment consisted of two or three periods (designed 0, F and S) during each of which the patients were given isocaloric diet containing 45 per cent of calories as carbohydrate (CHO), 35 per cent as fat (mainly saturated) and 20 per cent as protein. The only difference between the periods was in the quality of dietary carbohydrate. During *Period 0* the main carbohydrate was starch, which provided an average of 80 per cent of CHO calories. The rest constituted of approximately 25 grams of lactose and 20 grams of sucrose daily In the diet of Period F 75 or 80 grams/day of starch was exchanged for the same amount of fructose added to the basal diet as free substance (not incorporated into the foods). During the *Period S* starch was substituted for 75 or 80 grams of sucrose the daily consumption of which thus amounted to approximately 100 gram corresponding to 20 to 25 per cent of total calory supply The length of each dietary period varied between 10 and 20 days and their sequence was alternated in a random fashion. In patients with manifest diabetes the Period S was omitted and comparison was only mad between fructose-containing and low-sugar diets whil in the nondiabetic subjects all three regimens were instituted. Weight was recorded every day and if systematic deviations were noticed the calory content of the diet was increased or decreased respectively without changing the relative amounts of the three main constituents.

Plasma triglyceride concentration was recorded every second day (27) and at the end of each period plasma triglyceride turnover was determined by the endogenous ^{3}H-glycerol labeling technique (10) the details of which have been described in a previous paper (25). The slope of the plasma triglyceride-^{3}H radioactivity decay curve was analyzed by a digital computer to obtain the best fit for fractional rate constant, k. From this parameter the total turnover (production) rat V of endogenous plasma triglycerides was calculated by using the formula

$$V = k \times 0.45 \times S \times b \text{ (mg/kg/hr)}$$

where S is the mean plasma triglyceride concentration (mg/100 ml) during the test (four determinations in 8 hours) the factor 0.45 is derived from the assumption that plasma space represents the distribution volume of circulating triglycerides and forms 4.5 per cent of body weight. The factor b is a correction for the relative decrease of plasma volume on increase of relati e body weight (1).

RESULTS

The effect of interchange of starch and fructose was studied in ten subjects. The triglyceride transport data are presented in Table I, which shows that plasma triglyceride concentration, fractional transport rate and production rate were closely similar during the two dietary periods. The corresponding values for five nondiabetic subjects passing through all three experimental periods are demonstrated in Table II. It is seen that during the sucrose period both triglyceride concentration and production rate showed a rising trend as compared to the values during the other two diets but because of the small number of cases none of the differences was significant at 0.05 level. The individual responses of plasma triglyceride concentration to the three diets appear from Fig. 1 which includes two additional

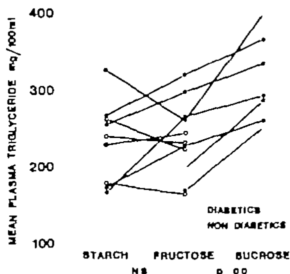


Fig. 1. Mean plasma triglyceride concentration during three dietary periods. Fructose and sucrose were administered 75 or 80 grams per day in an isocalori exchange for starch. Analyses were made every second day and thus the recorded values are averages from 5 to 9 individual measurements.

Table I. *Plasma triglyceride concentration and turnover (mean ± s.d.) during starch and fructose diets*

Diet	TG concentration	TG fractional turnover	TG production rate
n = 10	mg/100 ml	h^{-1}	mg/h/kg
Starch	206 ± 165	0.167 ± 0.070	14.3 ± 4.0
Fructose	258 ± 119	0.162 ± 0.042	14.4 ± 4.5

Table II. *Plasma triglyceride concentration and turnover (mean ± s.d.) rate in nondiabetic hypertriglyceridemic subjects durng three dietary periods*

Diet	TG concentration	TG fractional turnover	TG production rate
n = 5	mg/100 ml	h^{-1}	mg/h/kg
Starch	220 ± 48	0.180 ± 0.041	14.1 ± 2.1
Fructose	229 ± 36	0.172 ± 0.020	14.4 ± 2.3
Sucrose	270 ± 95	0.179 ± 0.044	16.8 ± 4.5

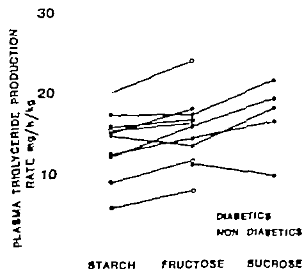


Fig. 2 Plasma triglyceride production (turnover) rate at the end of three dietary periods each lasting for 10 to 20 days.

subjects where fructose and sucrose were compared but the data of starch period had to omitted because the weight did not reach a steady-state level. The response to fructose was highly variable but it seems that most of the hyperglyceridemic patients without diabetes showed a definite rising trend of their plasma triglyceride level when transferred from starch to fructose diet. On the other hand, an increase of plasma triglyceride occurred in every subject when starch or fructose were exchanged for sucrose. With this number of cases the differences sucrose fructose and sucrose starch are not significant. Plasma triglyceride production rate in individual cases is illustrated in Fig 2. A slight trend to increase is apparent when fructose is compared with starch and when the sucrose period is compared with the non-sucrose ones but, again, there is no statistically significant difference in the triglyceride synthesis between the three periods.

DISCUSSION

The dietary sugar intake shows great individual and local variations but it usually ranges from 50 to 150 grams per day corresponding to 10 to 25 per cent of total calories (34). All those human studies where the hyperglyceridemic effect of sucrose has been demonstrated have employed more or less unphysiological diets with sugar covering 40 to 100 per cent of calories and amounting to 200 to 500 grams daily (2, 4 11—14, 19). With smaller sucrose intakes no effect on plasma triglyceride level has been observed in normoglyceridemic subjects (9 15) but in patients with type III or IV hyperlipoproteinemia *Little et al. (1970) reported* an increase of plasma triglyceride level when 20 per cent of calories was given as sucrose instead of starch. Low-sucrose and average sucrose (85 g/day) diets were compared in human volunteers by Mann et al. (20) who found significantly lower triglyceride levels with the former regimen. However the decrease coincided with weight loss and this makes the evidence for sucrose inconclusive.

The present study demonstrates that in subjects with primary endogenous hyperglyceridemia sucrose intake at an average level of 100 grams daily tends to increase plasma triglyceride concentration from the value present at low sugar (about 20 g/day) consumption. Under the controlled dietary conditions used here the increment remained relatively slight but on the other hand, no experience was gained on the behaviour of plasma triglyceride under a more prolonged feeding of sucrose at this intake level. It is known from earlier studies that the effect of high sucrose diet on serum lipids is dependent on age, sex and nature of dietary fat. Thus hyperglyceridemia is not induced by sucrose in premenopausal women (16) or in subjects eating much polyunsaturated fat (3). In these respects the patients of the present study should have been particularly sensitive

to sucrose since all were either men or post menopausal women and their dietary fat was mainly saturated. The resistance of pre-menopausal women to any hyperglyceridemic factor may be accounted for by their effective plasma triglyceride removal system (25)

As fructose is now being marketed at a reasonable price and it is advertised as a sweetener to replace sucrose, a comparison between these two sugars with regard to their effect on plasma triglyceride metabolism has become important. It is known that fructose readily induces hypertriglyceridemia in rats (26) and in man (13 17). In the study by Kaufmann and associates (13) fructose appeared to be slightly more hyperglyceridemic than sucrose. In the present series, on the other hand, fructose seemed to be slightly less active than sucrose in this respect but the number of subjects studied is too small and the differences are too slight to allow drawing of any definite conclusions at this stage of the study The present results also suggested that well controlled diabetics can tolerate a substitution of 80 grams of fructose for starch daily without any harmful effects on either blood glucose or plasma triglyceride The possible effect of an equal amount of sucrose on the control of diabetes and on plasma lipid levels in diabetic patients is not known, however

The metabolic effects of dietary starch, sucrose and fructose show differences which make it possible to explain the different actions of these carbohydrates on plasma triglyceride transport. The present kinetic data indicate that the fractional removal rate is not influenced by the nature of dietary carbohydrate and thus rules out the possibility that the sugars elevate plasma triglyceride by interfering with the triglyceride elimination from blood stream The changes observed in plasma triglyceride concentration are therefore wholly accounted f r by corresponding alterations in the rate of triglyceride secretion into plasma. It seems that this rate is lowest with starch diet and is increased with fructose but even more with sucrose. That fructose increases the plasma triglyceride concentration more than an equivalent amount of starch might be accounted for by the markedly greater hepatic uptake of fructose than glucose (22, 31) and by the increase of glycerol phosphate content of the liver after fructose load (6, 35) Of orally administered fructose a much higher proportion is converted to hepatic and plasma lipids than of dietary glucose (or starch) (18 21 23) and a similar difference in the incorporation of carbon from the two sugars into hepatic lipids is observed also in vitro (23 28, 36). A more extensive discussion on the possible explanations for these differences between starch and fructose (or sucrose) has been presented elsewhere (24)

The more marked stimulating effect of sucrose than of fructose on hepatic production of plasma triglycerides might be accounted for by the different responses of blood glucose, plasma insulin and FFA to sucrose and fructose. These three factors are currently believed to be important stimulators of plasma triglyceride synthesis (for references see 24) After sucrose load the magnitude of hyperglycemia, hyperinsulinemia and rebound increase of FFA are all more pronounced than after ingestion of similar amount of fructose (32). In the concentrations reached in peripheral blood after oral loads fructose does not discharge insulin from islet cells (7 8, 30) and as a consequence of the absent hyperinsulinism no hypoglycemia and concomitant rise of plasma FFA follows intake of fructose as occurs after sucrose. On the other hand, fructose itself inhibits the liberation of FFA from adipose tissue as effectively as glucose (29). In sucrose the metabolic effects of glucose and fructose are combined and this is particularly favorable for enhancement of plasma triglyceride synthesis. Thus, sucrose not only causes a rapid increase of the blood glucose and plasma insulin levels but simultaneously an excess of glycerol

phosphate is formed in the liver That all these factors effectively promote the synthesis and release of triglycerides by the liver is shown by the experiments of Topping and Mayes (33) where both fructose and insulin separately stimulate production of very low density lipoproteins by rat liver perfused with a high glucose medium.

ACKNOWLEDGEMENTS

This study was aided by grants from Finnish Food Research Foundation, Sigrid Jusélius Foundation and Finnish State Medical Research Council.

The authors are grateful to Mrs. Taru Wiherheimo, Miss Sirkka-Liisa Tolkka and Miss Hannele Suurinkeroinen for excellent technical assistance and to Mrs. Anneli Ollus, M.Agr.Sci. (Nutr) hospital dietitian, for controlling the diets.

REFERENCES

1 Alexander J K., Dennis, E. W Smith, W G Amad, K. H., Duncan, W C. & Austin, R. C. Blood volume cardiac output and distribution f systemic blood flow in extreme obesity Cardiovasc. Res. Bull. Baylor Univ 1 39 1962. Quoted in Medical Physiology Vol. I p. 250 C. V Mosby Comp., St. Louis, 1968.

2. Antar M. A., Little, J A., Lucas, C., Buckley G C. & Csima, A. Interrelationships between the kinds of dietary carbohydrate and fat in hyperlipoproteinemic patients. Part 3. Synergistic ffect of sucrose and animal fat on serum lipids. Atherosclerosis 11 191 1970

3. Bar-On, H. & Stein, Y Effect of glucose and fructose administration on lipid metabolism in the rat. J Nutr 94 95, 1968.

4 Barter P J Carroll, K. F & Nestel, P J Diurnal fluctuations in triglyceride free fatty acids, and insulin during sucrose consumption and insulin infusion in man. J clin. Invest. 50 583, 1971

5. Birchwood, B. L., Little, J A Antar M. A., Lucas, C., Buckley G C., Csima, A. & Kallos, A. Interrelationship between the kinds of dietary carbohydrate and fat in hyperlipoproteinemic patients. Part 2. Sucrose and starch with mixed saturated and polyunsaturated fats. Atherosclerosis 11 183, 1970

6. Bässler K H. & Stein, G Biochemische Grundlagen für Wirkungsunterschiede zwischen Sorbit und Fructose. Hoppe-Seyler's Z. Physiol. Chem. 348 533, 1967

7 Coore H. G & Randle, P J Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J 93 66, 1964

8. Creutzfeldt, W Frerichs, H. & Reich, U In vitro studies on rat islets. In The Structure and Metabolism of Pancreatic Islets, p. 321. Pergamon Press, Oxford, 1964.

9 Dunnigan, M. G Fyfe, T McKiddie, M. T & Crosbie, S. M. The effects of isocaloric ex change of dietary starch and sucrose on glucose tolerance plasma insulin and serum lipids in man. Clin. Sci. 38 1 1970.

10 Farquhar J W Gross R. C., Wagner R. M. & Reaven, G M. Validation of an incompletely coupled two-compartment non recycling catenary model for turnover of liver and plasma triglyceride in man. J Lipid. Res. 6 119 1965.

11. Hodges, R. E. & Krehl, W A. The role of carbohydrates in lipid metabolism. Amer J clin. Nutr 17 334, 1965.

12. Kaufmann, N A., Poznanski, R., Blondheim, S H. & Stein, Y Changes in serum lipid levels of hyperlipemic patients following the feeding of starch, sucrose and glucose. Amer J clin. Nutr 18 261, 1966.

13. Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y Effect of fructose, glucose, sucrose and starch on serum lipids in carbohydrate induced hypertriglyceridemia and in normal subjects. Israel J med. Sci. 2 715, 1966.

14 Kuo, P T & Basset, D R. Dietary sugar in the production of hyperglyceridemia. Ann. int. Med. 62 1199 1965.

15 Little J A., Birchwood, B. L., Simmons, D. A., Antar M. A., Kallos, A., Buckley G. C & Csima, A. Interrelationship between the kinds of dietary carbohydrate and fat in hyperlipoproteinemic patients. Part 1 Sucrose and starch with polyunsaturated fat. Atherosclerosis 11 173, 1970.

16. Macdonald, I The lipid response of young women to dietary carbohydrates. Amer J clin. Nutr 16 458 1965

17 Macdonald, I. Influence of fructose and glucose on serum lipid levels in men and pre- and postmenopausal women. Amer J clin. Nutr 18 369 1966.

18 Macdonald, I Ingested glucose and fructose in serum lipids in healthy men and after

myocardial infarction. Amer J clin N r 21 1366, 1968.

19. Macdonald, I. & Braithwaite, D M The influence of dietary carbohydrat lipid pattern in serum and in ad Clin. Sci. 27 23, 1964.

20. Mann, J I., Truswell, A. S H & Manning, F Effects on normal men of reducing di t starch for five months. Lan t 9 J

21. Maruhama, Y.. Conversion rb hydrate-[14]C into glycerol an f serum triglyceride in pati nts d infarction. Metabolism 19 10 1

22. Mendeloff, A. I. & Weichselbaum T Rol of the human liver in the a n tion f intravenously administered fr t se M tabolism 2 450, 1953.

23. Nikkilä, E. A. Modulat. f t gly eride kinetics by fructose versus glucose in the rat Scand. J clin. Lab. In est 18 ppl 9 8, 1966.

24. Nikkilä, E. A. Control f plasma and liver triglyceride kinetics by ca boh dr t metabolism and insulin Adv Lipid Res 7 63, 1969.

25. Nikkilä, E. A. & Kekki, M Polymorphism of plasma triglyceride kinetics in normal human adult subjects Acta med scand 190 49, 1971

26. Nikkilä, E. A. & Ojala, K Induction f hypertriglyceridemia by fructose in the rat Life Sci. 4 27 1965.

27 Noble, R. P & Campbell, F M Improved accuracy in automated fluorometric determination of plasma triglycerides Clin Chem 16 166, 1970.

28 Pereira, J N & Jangaard, N O Different rates of glucose and fructose metabolism in rat liver tissue in vitro. Metabolism 20 392, 1971.

29 Perry W F & Tjaden, B. J The effect of various saccharides on the release of nonesterified fatty acids from adipose tissue in vitro. Canad. J Biochem. Physiol. 40 455, 1962.

30 Pozza, G Galansino, G H ffeld, H. & Foà, P P Stimul tion of insulin output by monosaccharides and monosaccharide derivatives Amer J Physiol. 192 497 1958.

31. Renold, A. E Hastings, A. B. & Nesbett, F B Studies on carbohydrate metabolism in rat liver slices. II. Utilization f glucose and fructose by liver from normal and diabetic animals. J biol. Chem. 209 687 1954.

32 Swan, D C., Davidson, P & Albrink, M. J Effect of simple and complex carbohydrates on plasma non-esterified fatty acids, plasma-sugar and plasma-insulin during oral carbohydrate tolerance tests. Lancet 1 60, 1966.

33 Topping, D. L. & Mayes, P A. Direct stimulation by insulin and fructose of very-low-density lipoprotein secretion by the perfused liver Biochem. J 119 48 P 1970.

34 Walker A. R. P Sugar intake and coronary heart disease. Atherosclerosis 14 137 1971.

35 Wieland, O & Matschinsky F Zur Natur den antiketogenen Wirkung von Glycerin und Fructose. Life Sci. 2 49, 1962.

36 Zakim, D Herman, R. H. & Gordon, W C. J The conversion of glucose and fructose to fatty acids in the human liver Biochem. Med. 2 427 1969

Acta med. scand. Suppl. 542, pp. 229—235

THE SIGNIFICANCE OF SUCROSE IN PRODUCTION OF HYPERTRIGLYCERIDEMIA

N. A. Kaufmann and J. Kapitulnik

From the Department of Nutrition, Hebrew University — Hadassah Medical School, Jerusalem, Israel

Abstract. The relationship between endogenous and carbohydrate-induced hypertriglyceridemia (C HTG) is reviewed. Different effects of sucrose, glucose, fructose, and starch on serum lipid levels in patients with C HTG are demonstrated. The significance of these effects in patients with C-HTG and their relation to those in normal subjects is discussed.

There is much confusion as to the proper use of the term carbohydrate-induced lipemia. As originally defined by Ahrens et al. (2) it referred to those patients in whom feeding of a diet rich in carbohydrates resulted in an increase of serum triglycerides (S-TG). However S-TG levels of normal subjects may respond, at least temporarily to dietary carbohydrates in a similar fashion (1 9 24 32). In addition, in patients with hypertriglyceridemia (HTG), elevated S-TG levels are found on a regular diet, in which carbohydrates amount to only 40—50 per cent of the total calories, while on the same diet in normal subjects normal S-TG levels are found. Therefore, the term endogenous hypertriglyceridemia has been suggested for these patients (14, 33). Endogenous HTG indicates that the elevated S-TG are derived from de-novo synthesis, mainly by the liver. On the other hand, in exogenous HTG the elevated S-TG are derived directly from dietary fat. The term endogenous S-TG does not, however differentiate between S-TG originating from re-esterification of the free fatty acids liberated from adipose tissue by lipolysis and S-TG derived from de-novo synthesis of fatty acids from carbohydrates and other precursors. Thus, fatty acids for the synthesis of S-TG are not necessarily derived from carbohydrates, and, therefore, not all patients with endogenous HTG are necessarily carbohydrate-induced. However all patients with carbohydrate-induced hypertriglyceridemia (C-HTG) belong by definition to the group of endogenous HTG.

The classification of Fredrickson et al. (21) is not relevant to the above considerations. This classification, which is now used by almost all investigators and clinicians, refers to the kind of lipoprotein which transports the excessive amounts of serum lipids. However since Fredrickson's Type III and IV refer to increased levels of very low density lipo-

Table I. *The effect of a carbohydrate rich diet and a low-calorie diet on serum lipid levels in a patient with hypertriglyceridemia*

Diet	Weight (Kg)	Serum triglycerides (mg/100 ml)	Serum total cholesterol (mg/100 ml)
Ad lib.	74.0	2 710	652
Carbohydrate-rich [1]	74.0—72.5	664	339
Fat rich [1]	72.5	492	232
Ad lib.	66.8	2 150	740
Low-calorie diet [2]	66.8—61.8	169	227

1 Diets were administered for 4 weeks. Carbohydrates comprised 75 (mainly as starch) and fats 45 / (mainly as vegetable oil) of total calories. S-TG and TC values are averages of the whole period.

2 Diet consisted of 1 000 calories (30 % carbohydrates and 30 fat) and was administered for 7 weeks. S-TG and TC values are averages of the last 2 weeks.

proteins (VLDL) or to the appearance of an abnormal lipoprotein in Type III these types are included in endogenous HTG as well. Indeed, Glueck et al. (22) found only half of their patients with Type IV hyperlipemia to be carbohydrate induced. As to Type V in which an elevation of both VLDL and chylomicrons is found, at least part of the increase in S-TG is of endogenous origin.

The term endogenous HTG should therefore be applied to all patients with increased VLDL, normal or abnormal, regardless of the composition of the diet consumed. Abnormal insulin secretion (14 46) obesity (5) and alcoholism (28) may be the most important etiological factors. However disturbances of S-TG removal, as found in insulin deprivation (10) or on administration of certain drugs as steroids (11) are also included in the group of endogenous HTG On the other hand, C-HTG is a special case of endogenous HTG and includes patients in whom feeding of a high carbohydrate diet of more than 65 per cent of total calories, raises S-TG levels above those found in normal subjects on a similar diet. C HTG also includes patients with endogenous HTG in whom isocaloric restriction of carbohydrates to less than 40—50 per cent of total calories with no concomitant weight loss, results in a reduction of S-TG levels to below those found when the patients were on a normal diet. In this latter case S-TG levels need not necessarily be reduced to normal by carbohydrate restriction. In other words, endogenous HTG refers to the de-novo synthesis of excess S-TG regardless of the composition of the diet. The term C HTG applies to those patients in whom the de-novo synthesis of excess S-TG is a result of the amount of carbohydrates in their diet.

As an example of this approach the following case is presented. In a 55 year old moderately obese woman, S-TG level of 2710 mg/100 ml and total cholesterol (TC) of 652 mg/100 ml were found on routine examination, and on an *ad lib* diet. She was put on a diet of 75 per cent of calories from carbohydrates, however most of the carbohydrates were given as polysaccharides. Her S-TG decreased to 664 mg/100 ml and TC to 339 mg/100 ml. When fats were substituted isocalorically for carbohydrates a further decrease of S-TG occurred (Table I) After some years the patient was reexamined and again high lipid levels were found. This time she was treated for her obesity with a 1000 calorie reducing diet (30 per cent of calories as carbohydrates, 30 per cent as fats and 40

per cent as proteins). She lost about 3 0 kg and again S-TG and TC decreased. Thus in this patient by the latter treatment obesity was proven to be an important factor in maintaining her endogenous HTG However even without restriction of caloric intake and resulting weight loss, carbohydrate inducibility of HTG could be demonstrated

The production of HTG by dietary carbohydrates in C-HTG is not only dependent on their amount in the diet but on their quality as well (28, 29) In many patients with C HTG isocaloric substitution of sucrose for starch, usually without significant weight gain, caused a further increase of S-TG and TC. However in most cases S-TG and TC levels were also elevated on the starch diet (Table II). In one patient (L. J) elevated S-TG were found on the sucrose diet only while in another patient (W M) there was no change in S-TG levels when sucrose was substituted for starch. In this latter patient, though, feeding of fructose resulted in a marked elevation of S-TG

The effect of sucrose in increasing serum lipid levels especially TG is not peculiar to patients with C HTG It is also found in normal subjects (3 6 7 41) especially in men and postmenopausal women (13 35 36, 37). However this effect cannot be demonstrated in either all normal subjects (32) or in all patients with C HTG (27) On the other hand, in many patients with C-HTG this difference between the response of serum lipids to starch versus sucrose was much more pronounced than in normal subjects, and differences of S-TG levels between the two diets up to 900 mg 100 ml were found.

Feeding of fructose, but not of glucose, led to an increase of serum lipids in normal subjects simila to that following sucrose feeding (38) and more labelled fructose than glucose was incorporated into S-TG (40) Patients w th C HTG also responded to prolonged feeding of fructose with increases in S-TG levels and usually also of TC. The effect of fructose in raising S-TG was sometimes much more pronounced than that of

Table II. *Comparison of the effect of d etary starch and sucrose on serum lipids.*

	Days		S-TG (mg/100 ml ± S D.)		TC (mg/100 ml ± S.D.)	
Patient	Starch	Sucrose	Starch	Sucrose	Starch	Sucrose
G R.	36	28	905 ± 198	1 479[1] ± 872	373 ± 41	483[2] ± 78
V.S.	16	28	805 ± 112	1 332[2] ± 473	282 ± 48	339 ± 112
G.L.	11	9	723 ± 56	1 425[2] ± 202	402 ± 25	343 ± 41
S.C.	24	14	664 ± 170	1 807[2] ± 232	374 ± 67	402 ± 23
K.N.	23	25	623 ± 167	949[2] ± 214	278 ± 20	353[2] ± 39
P.S.	29	14	282 ± 62	1 164[2] ± 253	212 ± 28	278[2] ± 25
L.J	28	14	169 ± 34	286[1] ± 112	197 ± 13	223[1] ± 17
W.M.	21	35	234 ± 68	282 ± 43	186 ± 21	216[2] ± 14

[1] Difference significant t 0 05 > p > 0.01
[2] *Difference significant t p < 0 01.*
S-TG = Serum triglycerides TC = Total cholesterol.

Values are verages for the whole period. Carbohydrate content of diets 78—80 / of calories. Content of sucrose in sucrose diet. 68 of carbohydrates.

the diet and blood lipids in man. J Nutr 79 349 1963.

7 Antar M. A. & Ohlson, M. A. Effect of simple and complex carbohydrates upon total lipids, nonphospholipids and different fractions of phospholipids of serum in young men and women. J Nutr 85 329 1965.

8. Antar M. A., Little, J A., Lucas, C., Buckley G C. & Csima, A. Interrelationship between the kinds of dietary carbohydrate and fat in hyperlipoproteinemic patients. Part 3. Synergistic effect of sucrose and animal fat on serum lipids. Atherosclerosis 11 191, 1970.

9 Antonis, A. & Bersohn, I. The influence of diet on serum triglycerides in South African white and Bantu prisoners. Lancet 1 3 1961

10. Bagdade J D Porte, D Jr & Bierman, E. L. Acute insulin withdrawal and the regulation of plasma triglyceride removal in diabetic subjects. Diabetes 17 127 1968.

11. Bagdade, J D Porte, D Jr & Bierman, E. L. Steroid-induced lipemia. A complication of high-dosage corticosteroid therapy Arch. intern. Med. 125 129 1970.

1_ Bar-On, H. & Stein, Y Effect of glucose and fructose administration on lipid metabolism in the rat. J Nutr 94 95, 1968.

13. Beveridge J M., Jagannathan, S N. & Connell, W F The ffect of the type and amount of dietary fat on the level of plasma triglycerides in human subjects in the postabsorptive state. Canadian J Biochem. 42 999 1964.

14. Bierman, E. L. & Porte, D J Carbohydrate intolerance and lipemia. Ann. intern. Med. 68 926, 1968.

15 Bierman, E. L. Insulin and hypertriglyceridemia. Israel J med. Sci 8 303, 1972.

16. Blankenhorn, D H., Chin. H. P & Lau, F Y Ischemic heart disease in young adults. Metabolic and angiographic diagnosis and the prevalence of Type IV hyperlipoproteinemia. Ann. intern. Med. 69 21 1968.

17 Cohen, A. M. Fats and carbohydrates as factors in atherosclerosis and diabetes in Yemenite Jews. Amer Heart J 65 291 1963

18. Cook, G C Absorption products f D(—) fructose in man. Clin. Sci. 37 675, 1969

19 Cramer K., Paulin, S & Werko, L. Coronary angiographic f ndings in correlation with ge body weight, blood pressure serum lipids and smoking habits Circulation 33 888, 1966.

20 Fleischman, A. I., Hayton, T & Bierenbaum, M. L. Serum lipids and certain di tary factors in young men with coronary heart disease J Amer diet Ass. 50 11_, 196"

21. Fredrickson, D S Levy R I & Lees, R S Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. New Engl. J Med. 276 34, 94 148, 215, 273, 1967

22. Glueck, C. J Levy R. I. & Fredrickson, D. S Immunoreactive insulin, glucose tolerance and carbohydrate inducibility in Types II III, IV and V hyperlipoproteinemia. Diabetes 18 39, 1969

23. Havel, R. J Kane, J P Balasse, E. O Segel, N & Basso, L. V Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoprotein in normotriglyceridemic and hypertriglyceridemic humans. J clin. Invest. 49 2017 1970

24. Hatch, F T Abell, L. L. & Kendall, F E. Effects of restriction of dietary fat and cholesterol upon serum lipids and lipoproteins in patients with hypertension. Amer J Med. 19 48 1955

25 Heinl R. A., Levy R. I., Fredrickson, D S. & Gorlin, R. Lipid and carbohydrates abnormalities in patients with angiographically documented coronary artery disease. Amer J Cardiol. 24 178, 1969

26. Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y Changes in serum lipid levels of hyperlipemic patients following the feeding of starch, sucrose and glucose. Amer J clin. Nutr 18 261 1966.

27 Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y Effect of fructose, glucose sucrose and starch on serum lipids in carbohydrate induced hypertriglyceridemia and in normal subjects. Israel J med. Sci. 2 715, 1966.

28 Kudzma, D J & Schonfeld, G Alcoholic hyperlipidemia induction by alcohol, but not by carbohydrate. J Lab clin. Med. 77 384, 1971

29 Kuo P T & Bassett, D R. Dietary sugar in the production of hyperglyceridemia. Ann. intern. Med 62 1199 1965.

30 Kuo, P T Hyperglyceridemia in coronary artery disease and its management. J Amer Med. Ass. 201 87 1967

31 Kuo, P T Feng, L. V & Pamintaun, J Study of the nailfold capillaries in hypertriglyceridemia (Types III and IV hyperlipoproteinemia). Circulation 41 309 1970.

32 Lees, R. S. & Fredrickson, D S. Carbohydrate induction of hyperlipemia in normal man. Clin. Res. 13 327 1965.

33 Lees R. S & Fredrickson, D S. The differentiation of exogenous and endogenous hyperlipemia by paper lectrophoresis. J clin. Invest. 44 1968 1965.

34 Lopez-S, A., Krehl, W A., Hodges, R E. & Good, E. L. Relationship between food consumption and mortality from therosclerotic heart disease in Europe. Amer J clin. Nutr 18 361, 1966.

35. Macdonald, I. & Braithwaite D M. The influence of dietary carbohydrates on the lip d pattern in serum and in adipose tissue Clin Sci. 27 23, 1964.

36. Macdonald, I. The effects of various dietary carbohydrates on the serum lipids d ring five-day regimen. Clin. Sci. 29 193, 1965

37 Macdonald, I. The lipid response of post menopausal women to dietary carbohydrates Amer J clin. Nutr 18 86, 1966

38. Macdonald, I. Infl ence of fructose and glucose on serum lipid levels in men and pre- and post-menopausal women Amer J clin Nutr 18 369 1966

39 Macdonald, I. Interrelationship between the influences of dietary carbohydrates and fats on fasting serum lipids. Amer J clin Nutr 20 345 1967

40. Macdonald, I.. Ingested glucose nd fructose in serum lipids in healthy men and fter myocardial infarction. Amer J clin Nutr 21 1366, 1968

41 Malmros, H. Dietary prevention f therosclerosis. Lancet 1 94, 1970.

42 Nestel, P J Carroll, K. F & Havenstein, N Plasma triglyceride response to carbohydrates, fats and calori intake Metabolism 19 1 1970.

43 I kkila, E A Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin Ad Lipid Res. 7 63, 1969

44 Ostancova K Herda S. & Zvolankova, K. Dietary fat and dietary sugar Lancet 1 494, 1965

45 Quarf rdt S H Frank, A., Shames, D M., Berman. M & St mberg, D Very low density lipoprotein triglycerid transport in Type IV hyperlipoproteinemia and the ffects of car bohydrate-rich diets J clin I vest. 49 2281 1970

46 Rea en, G M Lerner R L Stern, M. P & F rquha J W R l of insulin in endogenous hypertriglyceridemia. J clin In est 46 1756 1967

47 White, L W & Landau, B R Sugar transport and fructose metabolism in human i testine in vitro J clin Invest 44 1200, 1965

48 Yudkin, J & Roddy J Levels of dietary sucrose in patients with occlusi e therosclerotic disease. Lancet 2 6, 1964

49 Zelis, R Mason, D T Braunwald E & Levy R I Effects of hyperlipoproteinemias and their treatment on the peripheral circulation. J clin Invest 49 1007 1970

the diet and blood lipids in man. J Nutr 79 349 1963.

7 Antar M. A. & Ohlson, M. A. Effect of simple and complex carbohydrates upon total lipids, nonphospholipids and different fractions of phospholipids of serum in young men and women. J Nutr 85 329 1965.

8. Antar M. A., Little, J A., Lucas, C., Buckley G C. & Csima, A.. Interrelationship between the kinds of dietary carbohydrate and fat in hyperlipoproteinemic patients. Part 3. Synergistic effect of sucrose and animal fat on serum lipids. Atherosclerosis 11 191 1970

9 Antonis, A. & Bersohn, I. The influence of diet on serum triglycerides in South African whit and Bantu prisoners. Lancet 1 3, 1961

10 Bagdade J D Porte, D Jr & Bierman, E. L. Acute insulin withdrawal and the regul tion of plasma triglyceride removal in diabetic subjects. Diabetes 17 127 1968.

11 Bagdade, J D Porte, D Jr & Bierman, E. L. Steroid induced lipemia. A complication of high-dosage corticosteroid therapy Arch. intern. Med. L.5 129 1970

12. Bar-On, H. & Stein, Y Effect of glucose and fructose administration on lipid metabolism in the rat. J Nutr 94 95 1968.

13. Beveridge, J .L, Jagannathan, S. N & Connell, W F The effect of the type and amount of dietary fat on the level of plasma triglycerides in human subjects in the postabsorptive state. Canadian J Biochem. 42 999 1964.

14. Bierman, E. L. & Porte D Jr Carbohydrate intolerance and lipemia. Ann. intern. Med. 68 976, 1968

15. Bierman, E. L. Insulin and hypertriglyceridemia. Israel J med. Sci 8 303, 1972.

16 Blankenhorn, D. H., Chin. H. P & Lau, F Y Ischemic heart disease in young adults. Metbolic and angiographic diagnosis and the prevalence of Type IV hyperlipoproteinemia. Ann. intern. Med. 69 .1, 1968

1 Cohen, A. M Fats and carbohydrates as factors in therosclerosis and diabetes in Yemenite Jews Amer Heart J 65 291, 1963

18. Cook, G C Absorption products of D(—) fructose in man. Clin. Sci. 37 675 1969

19 Cramer K., Paulin, S. & Werkö, L. Coronary angiographic findings in correlation with ge, body weight, blood pressure serum lipids and smoking hab ts Circulation 33 888, 1966

20. Fl ischman, A. I H..yton, T & B erenbaum, .L L. Serum lipids and certain dietary factors in young men w th coronary heart disease. J Amer d. t Ass 50 11.., 1967

.1. Fredrickson, D S Levy R I & Lees, R S Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. New Engl. J Med. 276 34, 94 148, 215 273, 1967

22. Glueck, C. J Levy R. L & Fredrickson, D. S.. Immunoreactive insulin, glucose tolerance and carbohydrate inducibility in Types II, III, IV and V hyperlipoproteinemia. Diabetes 18 739 1969

23. Havel, R. J Kane, J P Balasse, E. O Segel, N & Basso, L. V Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoprotein in normotriglyceridemic and hypertriglyceridemic humans. J clin. Invest. 49 2017 1970.

4. Hatch, F T Abell, L. L. & Kendall, F E.. Effects of restriction of dietary fat and cholesterol upon serum lipids and lipoproteins in patients with hypertension. Amer J Med. 19 48 1955

25 Heinle, R. A., Levy R. I., Fredrickson, D. S. & Gorlin, R. Lipid and carbohydrates abnormalities in patients with angiographically documented coronary artery disease. Amer J Cardiol. 24 178, 1969

26. Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y Changes in serum lipid levels of hyperlipemic patients f llowing the feeding of starch, sucrose and glucose. Amer J clin. Nutr 18 261 1966.

27 Kaufmann, N A., Poznanski, R., Blondheim, S. H. & Stein, Y.. Effect of fructose, glucose, sucrose and starch on serum lipids in carbohydrate induced hypertriglyceridemia and in normal subjects. Israel J med. Sci. 2 715, 1966.

28. Kudzma, D J & Schonfeld, G.. Alcoholic hyperlipidemia induction by alcohol, but not by carbohydrate. J Lab. clin. Med. 77 384, 1971.

29 Kuo P T & Bassett, D. R. Dietary sugar in the production of hyperglyceridemia. Ann. intern. Med. 62 1199 1965.

30 Kuo, P T Hyperglyceridemia in coronary artery disease and its management. J Amer Med. Ass. 201 87 1967

31 Kuo, P T Feng, L. V & Pamintaun, J Study of the nailfold capillaries in hypertriglyceridemia (Types III and IV hyperlipoproteinemia). Circulation 41 309 1970.

32. Lees R. S. & Fredrickson, D. S. Carbohydrate induction f hyperlipemia in normal man. Clin. Res. 13 327 1965.

33 Lees R. S. & Fredrickson, D S. The differentiation f exogenous and endogenous hyperlipemia by paper electrophoresis. J clin. Invest. 44 1968, 1965.

CONCLUDING REMARKS

Acta med. scand. Suppl. 542, pp 239—244

CONCLUDING REMARKS

E. R. Froesch

Metabolic Unit, Department of Medicine University of Zurich, Switzerland

First of all I wish to thank the scientific committee and in particular Dr Nikkilä and Dr Huttunen for the invitation to partic ipate in this very successful symposium. My thanks in the name of all participants go also to the Endocrine Society as the organizer of this symposium. I have long tried to figure why an endocrine society may want to or ganize a symposium on fructose This problem puzzles me particularly because I am right now president of the Swiss Society of Endocrinology and since I also have a major interest in fructose. I venture to give an explanation It must have to do with the sperm. The sperm belongs to the domain of the endocrinologist. Sperms do feed mainly on fructose and, as it seems, successfully and without any harm to their lipid metabolism. We may say without any exaggeration that none of us would be here if fructose had not been with us until conception.

Before going any further I would like to extend my thanks to the sponsor of the symposium, the Finnish Sugar Company which has provided so many goodies to make us feel happy and at home. They have successfully and convincingly demonstrated that nothing not even fructose, can substitute a well prepared, excellent dish of fish or meat, smoked or cooked.

Fructose is a very early acquaintance of men since he is incubated in a fructose containing medium already as a sperm. The fetus then looses the habit of consuming fructose. The newborn may again get in touch with it 1 or 2 days after birth. Most newborns tolerate sucrose and fructose very well. However a few among them are killed by fructose. Dr Perheentupa and Dr Scha pira have reported on hereditary defects of fructose metabolizing enzymes, the most frequent of which is hereditary fructose intol erance. Children with hereditary fructose intolerance may die within 1—2 months after birth if fructose or sucrose is present in their formula. Fructose does not harm children with fructokinase deficiency Also, patients with essential fructosuria do not present an increased incidence of atheroma tosis. Thus, increased blood levels of fruc tose by themselves do not harm blood vessels. It is of interest that only a few years

attention has focussed on the possibility that endogenously formed fructose and sorbitol may be causally related to atheromatosis. Aldosereductase and sorbitol dehydrogenase which convert glucose to sorbitol and fructose are present not only in the prostate and seminal vesicles to prepare the nutritional provision for the sperm for its journey to the ovum. Here these enzymes play an important role in reproduction. Their presence in the arterial and arteriolar wall, however may be harmful. At high blood sugar concentrations, these enzymes convert appreciable quantities of glucose to sorbitol and fructose. Sorbitol is trapped in these cells since there is no membrane carrier for this polyol. Sorbitol increases the intracellular osmolality leads to water uptake by the cell and to changes of the shape of cells and of the contacts between them. It is conceivable that swollen endothelial cells may loose touch so that lipoproteins may penetrate between cells and get trapped in the arterial [1] Diabetic arteriopathy may therefore, pathogenetically related to the endogenous of fructose and sorbitol from gluose Thus, it is possible that the enzyme system which makes life possible by providing fuel for the sperm kills men later on by promoting atheromatosis.

Let us return quickly to inborn errors of fructose metabolism. A new hereditary disease fructose-1 6-diphosphatase deficiency has been described by Baker and Winegrad. These children have frequent attacks of acetonemic vomiting in combination with hypoglycemia and most of them die in early infancy These children maintain normoglycemia during fasting as long as hepatic glycogen can be broken down and released by the liver as glucose. Once the glycogen stores are exhausted glucose can no longer be produced since fructose-1 6-diphosphate cannot be converted to fructose-6-phosphate due to the deficiency in fructose-1 6-diphosphatase Fructose leads to acute hypoglycemia, but in contrast to hereditary fructose intolerance without nausea and vomiting Therefore, these children do not develop a distaste for sweets. Fructose induced hypoglycemia is due to an inhibition of phosphorylase by fructose-1 phosphate and fructose-1 6-diphosphate at low intracellular concentrations of inorganic phosphorus.

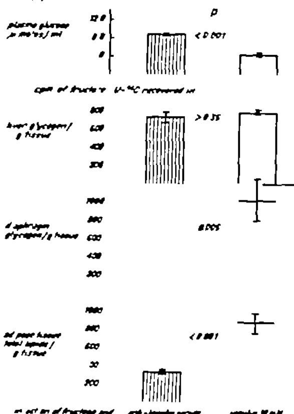


Fig. 1 Rats weighing 100 g were anaesthetized with Valium® 20 mg of fructose with 1 μCi of U ^{14}C fructose was administered intravenously together with anti-insulin serum or insulin. The rats were decapitated 30 minutes after the in travenous injection and the blood and tissues were analyzed for carbon-14. (From Froesch et al. Europ J clin. Invest 2 8, 1971)

I would like to put forward a plea to the manufacturers of baby food. Hereditary fructose intolerance is a frequent inborn error of metabolism. Many newborns with hereditary fructose intolerance are still being

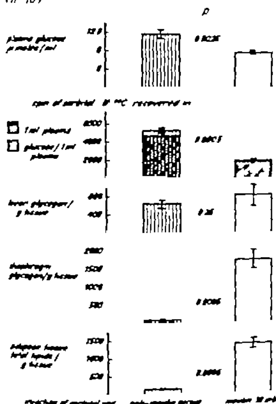


Fig. 2. Similar experiment as that shown in figure 1 with sorbitol instead of fructose (From Froesch et al Europ. J clin I vest 1 8, 1971)

killed by fructose in the first few months of their life. The diagnosis of hereditary fructose intolerance is difficult in newborns receiving fructose in their formula from birth on. Therefore, pediatricians should forcefully convey the idea to food manufacturers to make every effort to replace sucrose and fructose by glucose or maltose in baby food.

We have heard nothing about the use of fructose in parenteral nutrition. In many countries fructose is used as the main carbohydrate during and after surgery. The reasoning behind this therapy is the notion that fructose is metabolized in an insulin-independent fashion. This notion is based on a misunderstanding. It is true that the half life of fructose is short, i.e. approximately 3 minutes and that it is the same in diabetics as in normal subjects. We have heard during this symposium that fructose is taken up and metabolized by the liver. What is its fate? Oxidative processes in the liver are triggered by the synthetic and detoxifying functions and not by substrate availability. The latter determines only whether glucose, lactate free fatty acids or in the case of fructose infusion, fructose is used as the major fuel. Since the half life of fructose is so short, only a minimal portion of an intravenous load will be oxidized directly. Some fructose will be stored as glycogen and later released by the liver as glucose and some will be incorporated into total lipids. However the greatest portion of an acute fructose load is rapidly converted to glucose and released into the blood. Fructose conversion to glucose is readily detectable in an insulin deficient diabetic whose blood sugar increases rapidly after a fructose load. In normal subjects hyperglycemia after fructose does not occur due to compensatory insulin secretion. I wish to illustrate this in a slide (Fig 1). This experiment was carried out with normal rats which received a load of fructose together with fructose-^{14}C. One group was simultaneously injected with anti-insulin serum, whereas the other group was treated with insulin. What are the essential differences between the two groups attributable to the presence or absence of insulin? Plasma glucose is raised in the rats receiving anti insulin serum. Incorporation of fructose-^{14}C into liver glycogen is the same in both groups. The difference of plasma glucose can be accounted for by the effect of insulin on the incorporation of glucose-^{14}C originating in the liver from fructose-^{14}C into muscle and adipose tissue.

The next slide shows a similar experiment, this time carried out with sorbitol-^{14}C instead of fructose (Fig 2). The counts in the glucosazones and in plasma were also deter

quantities of fructose. I do not think that this very important question has been answered during this symposium to the satisfaction of all of us.

Future research will tell us whether fructose is really the crucial nutriment to make us feel that "life is so sweet on the sunny side of the street".



Index

Acta Medica Scandinavica

Supplementum 543

Family Studies in Systemic Lupus Erythematosus

By Rolf A. Larsen

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw kr. 170 incl. postage.
Other countries: Sw kr. 200 incl. postage.

Chief Editor

Professor Jan Waldenström, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell
Informationsindustri AB
S-751 81 Uppsala, Sweden

Family Studies in
Systemic Lupus Erythematosus

From Section of Immunology (Chief M. Harboe) Institute for Experimental Medical Research,
University of Oslo Ullevål Hospital, Oslo 1 Norway

Family Studies in Systemic Lupus Erythematosus

BY
ROLF A. LARSEN

OSLO 1972

Sats: Grafisk kontor NTNF
Trykk: Universitetsforlagets Trykningssentral, Oslo

Contents

The papers "Family studies in systemic lupus erythematosus (SLE) VI–IX. are published in Journal of Chronic Diseases.

Acknowledgements

The present investigation was carried out during the years 1965–1967 when I worked as a Research Fellow at the Institute for Experimental Medical Research, Ullevål Hospital, Oslo. During the years 1968–1969 I continued the work at the University of Oslo, Blindern, with a CD 3300 computer.

I am very grateful for the excellent working facilities provided by Professor F. Kiil, Head of the Institute for Experimental Medical Research, by Dr. H. Heisto and his staff at the Blood Bank, Oslo City Hospital, and by cand. real. P. Ofstad, the daily leader of the CD 3300 computer at the University of Oslo, Blindern.

Professor M. Harboe, Chief of Section of Immunology, Institute for Experimental Medical Research, was my teacher in immunology and I am most grateful for his valuable criticism and advice in the preparation of the manuscripts. I was very happy to be a member of the seminar group on immunology under his excellent leadership.

I was helped by Miss A. Vogt and Mrs. A. Deverill at the Institute, both of whom unhesitatingly solved many practical problems, by Miss A. Nannestad and her staff in the Medical Library, Ullevål Hospital, and by Miss J. Bonfield, who corrected the English and typed the manuscripts. To all, I express my sincere thanks.

I also wish to thank co-workers in this study Drs. A. Bøyum, B.G. Solheim, and T. Godal, for excellent co-operation.

I have been aided by financial support from Anders Jahre's Fund for the Promotion of Science, Norsk Forening til Kreftens Bekjempelse, Det Norske Revmatismeråd, and the Norwegian Research Council for Science and the Humanities, and to these institutions I express my gratitude.

To those not mentioned here by name, and to my family, all of whom have been of much assistance to me during the present work, I extend my warmest thanks.

Oslo, January 1972.

Rolf A. Larsen

Introduction

During the first half f this century t least 40 reports f familial discoid lupus erythematosus (DLE) appeared in the literature (for refe rences, see *Leonhardt* (8)) *Klemperer t al.* (2) in their autopsy protocol, noted family in which the mother had DLE and the daughter autopsy-proven systemic lupus erythematosus (SLE) (cited by *Dubois* (2))

SLE in identical twins was reported by *Davis & Gutridge* (1) but during the last twenty year only few new reports of SLE in twins have been published. Until 1966 ther were 46 complete and incomplet reports f familial SLE (for eferences, see *Leonhardt* (1) *Mass* (9))

Pedigree analyses around patients with SLE, performed by *Leonhardt* (7) and *Larsson & Leonhardt* (6) showed familial aggregation f hyp gammaglobulinemia. Hypergammaglobulinemia was shown to precede SLE, and this familial abnormality seemed to be an indication that SLE developed in genetically predisposed individuals. The familial aggregation f hypergammaglobulinemia was confirmed in more recent studies (for references, see *Mass* (9)) Most studies showed increased frequencies of antinuclear factors (ANF) rheumatoid factors (RF) hypergammaglobulinemia, and rheumatic diseases in relatives of patients with SLE ompared with ontrols, and multiple aberrations were shown in some families. *Morteo et al.* (10) demonstrated that three-quarters of their 19 families had t least one first degree relative with t least one abnormality *Pollak* (11) f und increased frequencies of ANF both in first and second degree latives of 43 patients with SLE, and the occurrence of familial SLE increased th frequency of ANF in the families

Most studies showed a wide spectrum of rheumatic diseases, but ANF RF and hyper gammaglobulinemia were also present in sera of apparently healthy family members. *Siegel t al.* (12) carried ut a systematic study in a defined population with proper controls, using index-case and index-control families and found increased frequency of hypergammaglobulinemia in the families of SLE probands. *Leonhardt* (8) studied index-case families in a defined area in Sweden, using the spouses of the relatives of the SLE probands as controls, and demonstrated statistically significant dif ferences between the relatives and the controls for several variables.

My family studies in SLE and SLE-like syndromes started bout 10 years ago when I first presented pedigree analysis including 57 members, around a forty-year-old woman with Hodgkin disease and symptoms resembling SLE (*Larsen* (3)) Pedigree analyses around SLE-like syndromes were reported in Lund (*Larsen* (4)) and both reports demonstrated aggregation of hypergammaglobulinemia and a variety f autoimmune disorder in the family members. A third report (*Larsen* (5)) including 15 first and second degre elatives around a patient with classical SLE, revealed that almost all members had one or more clinical and serological abnormality The serum of th mother of th SLE proband revealed antinuclear factors, including LE factor rheumatoid factors, thyroid antibodies and hypergammaglobulinemia, but sh was apparently healthy

Systematic and extensive index-case family studies in SLE – with the spouses of the relatives and the SLE probands used as ontrols – wer started in 1963 and the main purposes wer

Introduction

During the first half of this century t least 40 eports of familial discoid lupus erythematosus (DLE) appeared in the literatur (for ef rences, see *Leonhardt* (8)) *Klemperer t al.* (2) in their autopsy protocol, noted a family in which the mother had DLE and th daughter autopsy-proven systemic lupus erythematosus (SLE) (cited by *Dubois* (2))

SLE in identical twins was reported by *Davis & Cutridge* (1) but during the last twenty years only few new reports of SLE in twins have been published. Until 1966 ther wer 46 complet and incomplet reports of familial SLE (for references, see *Leonhardt* (1) *Man* (9))

Pedigree analyses around patients with SLE, performed by *Leonhardt* (7) and *Larsson & Leonhardt* (6) showed familial aggregation of hyp rgammaglobulinemia. Hypergammaglobulinemia was shown to precede SLE and this familial abnormality seemed to be an indication that SLE developed in genetically predisposed individuals. The familial aggregation of hypergammaglobulinemia was confirmed in mor recent studies (for references, see *Man* (9)) Most studies showed increased frequencies of antinuclear factors (ANF) rheumatoid factors (RF) hypergammaglobulinemia, and rheumatic diseases in relatives of patients with SLE compared with ontrols, and multiple aberrations were shown in some families. *M rtee t al.* (10) demonstrated that three-quarters of their 19 families had t least ne first degree relative with at least one abnormality *Pollak* (11) found increased frequencies of ANF both in first and second degree elatives f 43 patients with SLE, and the occurrence of familial SLE increased the f quency f ANF in the families.

Most studies showed a wide spectrum of rheumatic diseases, but ANF RF and hyper gammaglobulinemia were also present in sera of apparently healthy family members. *Siegel et al.* (12) carried out a systematic study in a defined population with p oper contr ls using index-case and index-control families, and found increased frequency of hypergammaglobulinemia in the families of SLE probands. *Leonhardt* (8) studied index-case families in a defined area in Sweden, using the spouses of the relatives of the SLE probands as ontr ls and demonstrated statistically significant dif ferences between the relatives and the controls for several variables.

My family studies in SLE and SLE-like syndromes started about 10 years go when I first presented a pedigree analysis, including 37 members, around a forty-year-old woman with Hodgkin disease and symptoms esembling SLE (*Larsen* (3)) Pedigree analyses around SLE-like syndromes wer reported in Lund (*Larsen* (4)) and both eports demonstrated aggregation f hypergammaglobulinemia and a variety of a toimmune disorders in the family members. A third report (*Larsen* (5)) including 15 first and second degree relatives ar und a patient with classical SLE, evealed that almost all members had ne or more linical and serological abnormality Th serum of the mother of the SLE proband evealed antinu lear factors, including LE factor rheumat id factors, thyroid antibodies and hypergammaglobulinemia, but she was apparently healthy

Systematic and extensive index-case family studies in SLE – with th spouses of the elatives and th SLE probands used as controls – were started in 1965 and th main purposes wer

a) to study clinical and serological aberrations in relatives of patients with SLE, and if familial aggregation were present to characterize the distribution in defined groups of relatives, including both first and second degree relatives,
b) to discover whether the aberrations in the relatives were related to the clinical findings in the SLE probands by classifying the patients with SLE according to the syndromes they exhibited during active stages of the disease
c) to investigate whether the aberrations in the relatives had a genetic basis.

The results of my studies are described in the 9 original papers listed in the Contents. An Editorial in the *Journal of Chronic Diseases* summarizes the results and presents a general discussion.

REFERENCES

1 Davi MW Gutridge GH: Disseminated l pus erythematosus in identical twin sisters associated with diabetes mellitus in one case J Missouri Med Ass 48: 446–450 1951
* Dubois EL: Chap 9 In Dubois EL Lupus Erythematosus. New York, McGraw Hill Book Company 1966
3 Larsen RA: L.E. cells in Hodgkin disease A f mily investigation Acta Path Microbiol Scand Suppl 154 196_a.
4 Larsen RA Family studies in SLE-l ke syndromes. (Orig In Norwegian) Trans Nord Foren Intern Med Lund 196*b p 46.
5 Larsen RA Family studies in systemic lupus erythematosus. (Orig in Norwegian) Nord Med 70: 1047 1963
6. Larsson O Leonhardt T: Hereditary hyper gammagl bulinemia and systemic lupus erythematosus. I. Clinical and electrophoretic studies. Acta Med Scand 165: 371–393 1959
7 Leonhardt T: Familial hypergammaglobulinemia and systemic lupus erythema tosus. Lancet ?: 1200–1*03 1957
8 Leonhardt T: Family studies in systemic lupus erythematosus. Acta Med Scand 176: suppl. 416 1964
9 Masi AT Chap 37 in Bennett PH Wood PHN: Population Studies of th Rheumatic Diseases. Amsterdam Excerpta Medica Foundation 1968.
10. Morteo OG Franklin EC, Ewen C, et al Clinical and laboratory tudies of relatives of patients with systemic lupus erythematosus. Arthritis Rheum 4: 356–365 1961
11 Pollak VE: Antinuclear antibodies in families of patients with systemic lupus erythematosus. New Eng J Med 271: 165–171 1964
1 Siegal M, Lee SL, Widelock D et al: Th epidemiology of systemic lupus erythema tosus. Preliminary results in New York City J Chron Dis 15: 131–140 196?
13 Larsen RA Editorial. Family studies in systemic lupus erythematosus. J Chron Dis. *5 187–190 1972.

I A proband material from central eastern Norway

ROLF A. LARSEN
Section for Immunology Institute for
Experimental Medical Research,
University of Oslo Ulleval Hospital, Oslo 1 Norway

ABSTRACT
A survey of SLE in an area from central eastern Norway is given, together with clinical and laboratory data on the 72 patients diagnosed as SLE in this area during the years 1959–1965. The main proportion of these cases would have been classified as definite SLE according to diagnostic criteria given in the literature. Annual incidence and prevalence rates were lower than rates presented from other countries. The frequency of various clinical manifestations is consistent with previous reports. The SLE patients were classified according to syndromes they exhibited during the active stages of their disease, and the manifestations in various syndromes were compared.

The SLE material serves as proband material for family studies, and sub-grouping of SLE cases is the basis for comparison of the findings in relatives of these various groups of SLE probands.

Since 1828 the concept of systemic lupus erythematosus (SLE) as a disseminated disease has gradually developed (for references see 26).

It has not yet been possible to recommend definite diagnostic criteria for SLE (20). The need for such criteria is urgent in the study of the epidemiology and the heredity of the disease. However SLE cases may be grouped according to the syndromes they exhibit during active disease (1).

The main purpose of the present paper is to describe a group of patients with SLE from central eastern Norway as a basis for discussion of the abnormal clinical and serological findings in the families of the patients.

METHODS AND CRITERIA

Material. The patients with SLE were collected from 6 fylker (shires) Akershus, Buskerud, Hedmark, Oslo, Vestfold and Østfold, in central eastern Norway with an area of 54,436 km and population of 1.43 million (4). About 54 per cent of the population lived in rural districts. Central eastern Norway has an entirely caucasoid population, with very stable residence.

All hospitals within the area were visited by the author who examined the patient registers for the years 1955–1965. The clinical and autopsy records of patients with a diagnosis of SLE (including suspected cases) and other collagen diseases were critically reviewed, and all LE cell preparations were re-examined. The patients were generally readmitted to the same hospital during the period. Patients admitted to two or more hospitals inside the area were examined for change in diagnosis. Suspected SLE cases were followed up for later admission to other hospitals. Patients with definite SLE diagnosed prior to 1959 were included in the material, if they were still alive after 1959. Fifty-one (70.4 per cent) of the 72 patients accepted as probands with definite SLE were personally examined by the author.

All available data relevant for diagnosis of SLE according to the literature were considered. Combinations of three or more of the following criteria were crucial for the diagnosis of SLE: intermittent fever, polyserositis, myocarditis, nephritis, pulmonary lesions, lupus skin lesions, skin hemorrhages, numerous LE cells, thrombocytopenia, leucopenia and hypergammaglobulinemia. However numerous LE cells (at least 15–20 LE cells per slide) was most often decisive. The patients were excluded mainly on the grounds of negative LE cell preparations. Negative autopsy findings in patients with a history otherwise typical of SLE did not exclude a diagnosis of SLE, nor did negative LE cell preparations, if necropsies were consistent with SLE (8). The syndromes which

are usually not considered as being typical for SLE, such as lupoid hepatitis, rheumatoid arthritis with LE cells and few systemic manifestations, or drug induced SLE, were excluded. Generally clinical manifestati ns alone did not sustain the SLE diagnosis. Thus the present SLE material consists of 4 different groups of patients patients with classical SLE including numerous LE cells patients with symptoms compatible with both SLE and rheumatoid arthritis including positive LE cell tests, patients with SLE and chronic discoid skin lesions and lastly patients with clinical and pathological SLE manifestations (wire loops periarterial fibrosis of the spleen or hematoxylin bodies) but with negative LE cell preparations.

The severity of SLE makes it likely that the patients at some time during the disease had to be hospitalized (73) but some benign cases of SLE are probably not admitted to hospital. Thus one male case was diagnosed by the author during his family studies, he had a titre of 1/8 in the indirect LE fact r test (vide infra) transient nephritis, serositis and fever and responded well to corticosteroid therapy Generally the diagnosis of SLE was made in the hosp t l in th are when the patients exhibited systemic manifestations, but a few patients would oid th present study primarily if the diagnosis of SLE or another collagen disease was not taken into consideration in hospitals; se ondly if they la ked sufficient laboratory dat Th fa il ties for hemat logical and histological n estigations varied in the hospitals.

Blood spec men f r LE factor determination has rout n ly been sent to th author from h spit l inside the rea after 1964 but dditional d fin t case from the period 1959 1963 w discovered from this material.

A tot l f 165 different diagnostic criteria wer summed p from nine papers (6 9 13 16 19 1 5 7) where varying numbers and we gh f sign fic anc were att ched t these n eria Th probands with SLE were ompared w h and la sif ed according to diagnos ria g en in b f th se 9 papers (6 9 13 (100 per ent) would have been j d defini SLE patient by the criteria given f hese r ferenc e papers, 69 (75 per en b h riteria given in 5 of th p pe d 4 5 per ent) by the cr eria gi en all pers 5 of the nine SLE cases which did not fulfill the criteria in all six papers would have been excluded by the criteria in one paper (13) mainly due to different views concerning the significance of necropsy findings and the LE cell phenomen Only one case would have been refused simultaneously by the criteria given in two papers.

Statistical methods The data were punched on data cards and processed in a CD 3300 computer at the University of Oslo Norway χ^2 calculations were performed when two subgroups of SLE patients were compared using 2 x 2 tables and one degree of freedom A continuity correction wa used (7) P-values below 0 05 were regarded as significant.

Criteria:

(a) *Hematological changes.* (1) Anemia hemoglobin concentrati n below 11.0 g per cent. ( ) Leucopenia: white blood cell count below 3000 per mm^3 (3) Thrombocytopenia: blood platelets below 150 000 per mm^3 (4) Numerous LE cells: at least 15–20 LE cells per slide using the direct techniques (1 8) (5) Few LE cells less than 15 LE cells per slide and extracellular material (ECM = LE bodies = globs) largely absent (6) Few LE cells and ECM less than 15 LE cells per slide but abundant ECM. (7) ECM ECM present but LE cells absent (8) Positive Waaler test a titre of at least 1/40 (9) Positive acryl particle test a ++ reaction at dilution 1/10 or higher (10) Hypergammaglobulinemia: gammaglobulin concentrations above normal mean + 4 SD the normal mean and SD were determined in the different laboratories where the paper electrophoresis tests were carried out by previous tests on healthy blood donors. Determination of IgG concentration in serum by th single radial diffusion technique has been described elsewhere (10)

(b) *Clinical syndromes:* (1) Lupus nephritis the patients with SLE were defined as having lupu nephritis when both the urinary sediment and tests of renal function were abnormal. ( ) Acute lupu kin lesions the following conditions were included in this term butterfly rash or eruption, nail bed changes, palmar erythema, photosensitiv ty and maculopapular lesion f lupus nature (cf Table V) (3) Chronic lupus kin les on both localized and generalized discoid lupu were regarded. (4) Marked arthr

tis the patients with SLE were defined as having marked arthritis when they had t least 5 of the American Rheumatism Associati n s criteria f r rheumat id arthritis (RA) (3)

A. Annual incidence and prevalence rates f SLE

For th calculation f incidence and prevalence rates, only patients living and diagnosed inside the area were onsidered, the rates being adjusted for increase of population (24) Two patients excluded from th SLE material were included when calculating the rates: one patient refused t cooperate for the family study the other was physician who was not informed about the diagnosis of the disease from which he was suffering. Tw female patients in the SLE proband material resided outside the area and were n t included when calculating th rates.

The annual rates of newly diagnosed definite SLE cases in the years 1959—1963 are given in Table I.

SLE patients diagnosed prior to and still living in 1959 were computed in the prevalence rate. Forty-six new definite SLE cases were diagnosed within th area in the period 1959—1963 The incidence per million varied from 4.1 in 1961 to 10.3 in 1962 The prevalence rate gradually increased from 19.6 per million in 1959 t 36.5 per million in 1963 The Zinkham-Conley test (28) was employed by more hospitals after 1961 and the increased incidence was mainly due t th introduction of this LE cell test. The gradual increase of prevalence of SLE during the period was a consequence f the increased incidence but was also due t longer survival f the patients.

Table I Incidence and prevalence rates of SLE in central eastern Norway

Year	N f new patients with diagnosis SLE	Incidence	Prevalence
1959	8	5.6	19.6*
1960	7	4 9	22.2
1961	6	4 1	25.5
1962	15	10 3	31.5
1963	10	7.5	36.5

* No of cases per million.

B. The ourse and duration f disease

The sex ratio was about 1 6 11 (15 3 per cent) of the 72 patients being males and 61 (84.7 per cent) females. The age at onset, t diagnosis, t death, and t sample study are presented for the two sexes in Table II.

Table II Age distribution of 72 patients with SLE

	No. of patients/Decades							
	0 – 9	10 – 19	20 – 29	30 – 39	40 – 49	50 – 59	60 – 69	>70
Females								
At onset	1	3	14	17	14	10	2	
At diagnosis		3	7	10	21	12	6	2
At death		1	4	6	7	1	6	
At sample study		1	3	7	17	10	5	1
Males								
At onset		2	3	1	2		3	
At diagnosis		1	3	1	2	1	3	
At death			1	2		1		
At sample study		1		1	2	3		

The age at onset refers to when the patients first exhibited manifestations which logically developed into a more definite clinical picture (4) and the age at diagnosis refers to the time when available data fulfilled the diagnostic criteria for definite SLE.

Females: 45 (70.8 per cent) of the 61 patients had their first symptoms in the third to the fifth decade and the main proportion (70.5 per cent) were diagnosed in the fourth to the sixth decade ?5 (40 9 per cent) died during the present study i.e., up to 1966

Males The age at onset and diagnosis was more widely distributed than in females. Four (36.4 per cent) of the 11 male patients died during the period of study

Eleven (15.3 per cent) of the 7? patients experienced an acute onset of the disease the diagnosis being established within 6 months, in ?1 cases the diagnosis was made within 18 months. Most patients experienced a gradual onset of the disease with an interval between onset and diagnosis of three years or more Generally the prognosis was poorer in cases diagnosed within years. Fourteen (19 4 per cent) of the 7? p tients died within 18 months of diagnosis.

The figures a e shown in Table III.

C. Cl ical manifestation

SLE s a chronic disseminated disease with tendency to exacerbations and remissions (8) Most organs of the body are affected. Gene rally gradual onset develops into an active

T le III Duration of disease in 72 patient with SLE

Months	From onset to diagnosis		From diagnosis to death	
	Female	Male	Female	Male
0 6	9		4	
7 18	7	3	8	
19 36	13	1	3	
37 60	6	1	5	1
61 84	6	1	3	1
85 106	8	1	1	
107 132	3	1	1	
>133	9	1		

phase followed by remission. Relapses of varying intensity and remissions, usually occur prior to and after the diagnosis of SLE.

The freq ency of various clinical manifestations seen in the 72 SLE patients is given in Table I\ The incidence at onset and at diagnosis is shown together with the cumula the incidence

Acute renal insufficiency was present in 11 patients (15.3 per cent) at the time of diagnosis, and the cumulative percentage was ?9.. Generally chronic renal failure developed after repeated or severe acute attacks, uremia occurred in 18 of the cases. Hematuria and proteinuria without renal insufficiency were found in higher percentages. Pulmonary lesions were observed in about 3/4 of the patients. Eleven (15.3 per cent) of the 72 SLE patients had thyroid disease hyperthyroidism 4 colloid goiter 3 non-toxic adenoma ? hypothyroidism 1 and subacute thyroiditis 1

The frequency of various clinical manifestations usually attributed to vascular lesions (4) is seen in Table V

Acute lupus skin lesions were present at onset in 13 (18.1 per cent) and chronic kin lesions in 4 (5 6 per cent) of the patients. Usually the skin lesions were of the same kind throughout the course in each patient. Except for more disseminated chronic forms, chronic discoid skin lesions ran their own course independent of variations in systemic involvement. This was observed in 6 probands, usually when the chronic discoid forms were localized to cheeks. Raynaud phenomena were an early and predominant feature in 11 of th 7? patients. Si of the 19 patients with Rayna d phenomena died of renal failure «Cytoid bodies wer diagnosed in single case

D Hemat l gical findings

A survey of hemat logical findings is presented in T ble VI. Percentages are shown for the number of patient tested.

Anemia wa present in 48 (66.7 per cent) f the patients, leucopenia in 20 (27 8 per cent) and thrombocytopenia in 18 (5 per cent)

Direct LE cell tests (all phenomena in cluded) were positive in all but two p tients who were tested in uremic phase A topsy rev aled typical wire loops in both these p

Table IV Frequency of various clinical manifestations in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
Fever > 38.5 C	16 (22.2**)	50 (69.4)	61 (84.7)
Intermittent fever	15 (20.8)	39 (54.2)	47 (65.3)
Myocarditis	0	10 (13.9)	18 (25.0)
Pericarditis	0	19 (26.4)	30 (41.7)
Nephritis (acute)	1 (1.4)	11 (15.3)	21 (29.2)
Proteinuria	2 (2.8)	36 (50.0)	50 (69.4)
Hematuria	1 (1.4)	21 (29.2)	29 (40.2)
Casts	0	12 (16.7)	19 (26.4)
Uremia	0	5 (6.9)	18 (25.0)
Hepatomegaly	2 (2.8)	7 (9.7)	20 (27.8)
Splenomegaly	1 (1.4)	8 (11.1)	12 (16.7)
Lymphadenopathy	4 (5.6)	19 (26.4)	30 (41.7)
Severe anorexia	11 (15.3)	17 (23.6)	27 (37.5)
Pleurisy	2 (2.8)	38 (52.8)	49 (68.1)
Pulmonary lesions	3 (4.2)	28 (38.9)	56 (77.8)

N of patients with the symptom.

** Percentage of patients with the symptom.

Cumulative incidence refers to the time of sample study or death.

Table V Frequency of clinical manifestations attributed to vascular lesions in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
Butterfly rash	2 (2.8)	6 (8.3)	11 (15.3)
Butterfly eruption	0	6 (8.3)	7 (9.7)
Nail bed changes	0	2 (2.8)	4 (5.6)
Palmar erythema	4 (5.6)	5 (6.9)	6 (8.3)
Photosensitivity	5 (6.9)	10 (13.9)	13 (18.1)
Maculopapular lesions	2 (2.8)	16 (22.2)	23 (31.9)
Discoid lupus – localized to cheeks	3 (4.2)	2 (2.8)	3 (4.2)
Discoid lupus – generalized	1 (1.4)	5 (6.9)	7 (9.7)
Alopecia	3 (4.2)	5 (6.9)	7 (9.7)
Purpura, ecchymosis	3 (4.2)	15 (20.8)	23 (31.9)
Urticaria	6 (8.3)	9 (12.5)	16 (22.2)
Raynaud phenomena	8 (11.1)	15 (20.8)	19 (26.4)
Neuritis	0	7 (9.7)	11 (15.3)
Arteritis of central nervous system	1 (1.4)	3 (4.2)	6 (8.3)
Epilepsia	1 (1.4)	1 (1.4)	5 (6.9)
Cytoid bodies	0	0	1 (1.4)
Hemorrhages (ocular fundic lesions)	0	3 (4.2)	4 (5.6)

The figures are recorded as in Table IV

Table VI Frequency of hematological aberrations in 72 patients with SLE

	At onset	At diagnosis	Cumulative incidence
Anemia	15 (21.1)	37 (51.4)	48 (66.7)
Hemolytic anemia	0	6 (8.3)	7 (9.7)
Leucopenia	1 (1.4)	11 (15.3)	20 (27.8)
Thrombocytopenia	0	12 (16.7)	18 (25.0)
Numerous LE cells	1 (2.4)	51 (71.8)	60 (83.3)
Few LE cells	1 (2.4)	3 (4.2)	3 (4.2)
Few LE cells and ECM	0	3 (4.2)	2 (2.8)
ECM	0	(2.8)	5 (6.9)
Waaler test positive	0	9 (13.6)	14 (19.6)
False lues serology	5 (6.9)	14 (19.6)	14 (19.6)
Hypergammaglobulinemia	2 (4.7)	34 (47.9)	36 (50.7)
Positive antiglobulin test	0	7 (13.5)	10 (13.9)
Cold agglutinins	0	1 (1.9)	5 (6.9)

The figures are recorded as in Table IV

tients. Numerous LE cells were seen in 60 of the cases. The direct LE cell tests were only weakly positive in 10 of the patients but 5 of these had titres of 1/4 or higher in the indirect LE factor test. The indirect LE factor test was positive in 39 (76.5 per cent) of the 51 patients with SLE personally examined by the author However the disease was not necessarily active during blood sampling. This test is described in detail in a separate paper (11) One patient with only extracellular homogeneous material (ECM) had a titre of LE factor of 1/64 by the indirect test and above 1/1000 of ntinuclear factors detected by immunofluoresc

The gamma-globulin co deter mined by paper el greatly increased in 36 of the pat normal mean value + 4 SD) It decr slightly during remissi ns.

*E. S*y *ry in SLE sync*

The were clas sy during the various then nts with a specified

Lupus rd of u developed forms)

The following variables were significantly increased in this group lymphadenopathy ($p = 0.0324$) skin hemorrhages ($p = 0.0398$) butterfly rash and eruption ($p = 0.008$) intermittent fever at diagnosis ($p = 0.0\ 4$) onset of SLE before menopause ($p = 0.017$) and mortality ($p < 0.001$)

SLE patients without lupus nephritis had significantly increased: myocarditis at diagnosis ($p = 0.04$) bronchitis ($p = 0.0242$) roentgenographic evidence of erosive arthritis at diagnosis ($p = 0.02$) and IgG concentrations ≥ 1740 mg/100 ml (p 4)

Hypergamm *linemia.* Thirty-six (50.7 per cent) of patients examined had hypergammagl This group of pa tients was ch d by severe symptoms of onset giving picture with high fever pleurisy pulmonary ir tions and of ARA c for rheumatoid Generally al ld symptoms distribut nts with and w agi

Acute T (28.9 per cent) t cute skin lesi

The f ntly p athy 0 0 lupus ages

Table VII. Frequency of rheumatic diseases in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
2 of ARA's criteria for RA	17 (23.7)	18 (25.0)	12 (16.5)
3–4 of ARA criteria for RA	19 (26.4)	21 (29.2)	27 (37.5)
5–6 of ARA criteria for RA	6 (8.3)	16 (22.2)	17 (23.6)
7–8 of ARA criteria for RA	0	5 (6.9)	7 (9.7)
Roentgenological changes in joints	3 (4.6)	13 (19.7)	18 (25.0)
Keratoconjunctivitis sicca	1 (1.4)	5 (6.9)	6 (8.3)
Effusions in small joints (toes and fingers)	24 (33.3)	24 (33.3)	45 (62.5)
Effusions in large joints (wrists, elbows, shoulders, knees and ankles)	28 (38.9)	29 (40.3)	50 (69.4)
Diffuse muscular pains	13 (18.1)	29 (40.3)	33 (45.8)
Ankylosing spondylitis	0	0	1 (1.4)
Rheumatic fever	5 (6.9)	0	12 (16.7)
Gout	1 (1.4)	2 (2.8)	3 (4.6)

The figures are recorded as in Table IV

Patients without cut skin lesions f lupus nature had increased frequency of effusions in small ($p = 0.02$) and large ($p = 0.009$) joints at diagnosis.

Marked arthritis. One-third f the SLE cases had at least 5 f ARA criteria for RA besides the lupus features. The frequency of rheumatic diseases in the 72 SLE patients is given in Table VII.

Initially roentgenological evidence f erosive arthritis was found in 3 (4.6 per cen) of the 72 patients, but these patients later developed clinical pictures consistent with definite SLE. However all ran a favourable course except for their arthropathy About one-quarter of the SLE cases had swellings in small joints (toes and fingers) both t onset and at diagnosis. The cumulative percentage of small joint effusions was 62.5 Effusions in joints were more fre quent prior t than after SLE diagnosis, largely due to orticosteroid therapy

SLE patients who developed marked arthritis had significant increase of th following variables ffusions in small joints prior to ($p = 0.0006$) and after SLE diagnosis (p 0.0396) effusions in large joints (wrists, lbows, shoulders, knees and ankles) prior to (p 0.0016) and after SLE diagnosis ($p = 0.0015$) Rayna d phenomena t diagnosis ($p = 0.005$) positive acryl particle test ($p = 0.015$) and positive Waaler test ($p < 0.001$)

SLE patients without marked arthritis had significant increase in the variables Intermittent fever t onset ($p = 0.0312$) and acute nephritis prior to diagnosis ($p = 0.018$)

F Cause f death and autopsy findings

Of th 72 patients, 29 (40.3 per cent) died during the present study mainly of renal insufficiency One female patient died in the active stage of SLE, but autopsy did not reveal renal damage.

Infectious diseases (pneumonia, septicemia and meningitis) were regarded as the cause f death in 9 patients. Four died of cardiac failure.

Autopsy was performed in 18 patients with the f llowing findings: wire-loops (88.9 per cent) onion kin lesions (77.8 per cent) fibrinoid ecrosis (44.4 per cent) vasculitis (44.4 per cent) myocarditis (27.8 per cent) hematoxylin-bodies (22.2 per cent) Libman-Sacks endocarditis (16.7 per cent) and amyloidosis (5.6 per ent)

DISCUSSION

There is no general agreement on what SLE is, and the diagnostic criteria are arbitrary (23) In the present study no definite criterion was employed, and all available data relevant for diagnosis of SLE according to data given in the literature were considered. Syndromes which were not regarded as typical SLE were excluded. The 72 patients given the diagnosis SLE were compared with and classified according to diagnostic criteria given in 6 papers (6 9 13 22 25 27):72 (100 per cent) would have been accepted as definite SLE patients by the criteria given in 3 out of these 6 references papers, 69 (95.8 per cent) by the criteria in 5 of the papers, and 63 (87.5 per cent) by the criteria given in all six papers. Six of the latter 9 SLE cases would have been excluded by the criteria used in one paper (13) mainly due to a different view concerning the significance of necropsy findings and the LE cell phenomenon.

The method of c llecting the SLE cases was based on the assumption than the difficulty of diagnosis and the clinical severity of SLE would lead to hospitalization at some time (23) This was apparently not always the case. Thus, one male patient who had not been hospitalized was diagnosed as SLE by the author during the family studies. This will contribute to the fact that the rates reported are in reality too low

When th ondition of the SLE patients deteriorates, the diagnosis of SLE depends upon th diagnostic criteria used and on how often the disease is considered likely Generally the diagn sis of SLE was considered when the patients exhibited systemic manifestations, but the facilities fo hematological and histological investigations varied from hospital to hospital. When the Zinkham-Conley test (28) – which is a sensitive and eliable LE ell test (3) – was employed by more hospitals th incidence of new SLE cases increased markedly

Th annual incidenc and prevalence rates of definit SLE calculated for entral eastern Norway in 1959–1965 wer lower than the rates previously eport d f m other ountries (13 18 22 23)

SLE is generally ognized as hr nic disseminated disease with tendency to exac erbations and remissions In th present study symptoms and igns t different phases f th disease were computed separately but a cumulative incidence was also calculated.

By lassifying SLE cases according to the clinical syndromes they exhibit during active disease a better understanding of the factors involved in different phases of the disease may be obtained. Fo example i SLE patients who developed lupus nephritis had increased fre quency of skin hemorrhages, butterfly rash or eruption the onset of SLE was before menopause fever was marked at the time of diagnosis of SLE and the mortality was high, but they had decreased frequency of bronchitis and myocarditis and roentgenological evidence of erosive arthritis at diagnosis of SLE and the IgG concentrations in serum were lower ii: SLE patients with hypergammaglobulinemia more often had a severe onset with a clinical picture of high fever pleurisy myocarditis and pulmonary infiltrations. iii The SLE patients who developed marked arthritis during the disease differed in several respects from the patients who did not become arthritic.

A search for concordance or discordance of the variables at different phases of the disease appears to be valuable Thus, in many cases, some of th lupus features, including organ involvements and laboratory findings, seemed to run their own course independent of each other Except for more disseminated chronic forms, chronic discoid skin lesions ran their own ourse independent of variations in syste mic involvement. The disease of patients with sless specifics criteria, like discoid skin lesions, marked arthritis and myocarditis, or chronic biologic false-positive test for syphilis, hemolytic anemia and purpura hypergammaglobulinemica, often ran a favourable rather chronic ourse

The symptomatology in the presently described patients with SLE is consistent with previous reports (2 for references, see 4 12) However the figures given in the literature vary The natural history of SLE in Norway also onfirms previous reports (14 15) but no sex difference in th prognosis was observed in the present study

The present material serves as proband material for family studies in SLE. The main purpose of this work was to investigate the linical and ser logical aberrations in families of SLE patients. Th description of SLE given

here is used as basis for the discussion of the abnormal findings in th families of the patients.

REFERENCES

1 Burnham T.K.: Lancet 1 666 1966.
2. Carpenter R.R. & Sturgill, B.C. J chron. Dis. 19 117 1966.
3 Committee of the American Rheumatism Association 1958 Revision of Diagnostic Criteria for Rheumatoid Arthritis. Bull. rheum. Dis. 9 : 175 1958.
4 Dubois, E.L. In Dubois, E.L., ed. Lupus Erythematosus, pp 129–276 McGraw-Hill Book Company New York 1966.
5 Dubois, E.L. In Dubois, E.L., ed.. Lupus Erythematosus, pp. 302–331 McGraw-Hill Book Company New York 1966
6 Dubois, E.L. In Dubois, E.L., ed. Lupus Erythematosus, pp. 332–339 McGraw-Hill Book Company New York 1966
7 Dunn, O.J : Basic Statistics A Primer for the Biomedical Sciences pp 121–125 J hn Wiley & Sons, Inc., New York 1964.
8 Harvey A.M., Shulman, L.E., Tumulty P.A., Conley C.L. & Schoenrich, E.H. Medicin (Baltimore) 33 291 1954
9 Kellum, R.E. & Haserich, J.R. Arch. intern. Med. 113 200 1964
10 Larsen, R.A. J chron. Dis. 25 205 1972
11 Larsen, R.A. & Boyum, A. Acta med. scand. Suppl. 543 p. 21 1972
12. Larson, D.L. Systemic Lupus Erythematosus. J & A. Churchill, Ltd., London 1961
13 Leonhardt, T Acta med. scand. Suppl. 416 1964
14. Maddock, R.K., J J Amer med. Ass. 191 137 1965
15 Merrell, M. & Shulman L.E. J chron. Dis. 1 12 1955
16 Muehrck R.C., Kark, R.M., Pirani, C.L. & Pollak, V.E. Medicine (Baltimore) 36 1 1957
17 N than, D.J & Snapper I : Blood 13 883 1958
18 Nobrega, F T Ferguson R.H., Kurland, L.T & Hargraves, M.M. In Bennett, P.H. & Wood, P.H.N eds. Population Studies of the Rheumatic Diseases, Proceedings of the third international symposium New Y rk, 1966 pp 259–266 International Congress Series No. 148 Excerpta Medica Founda-ti n, Amsterdam 1968
19 Noonan, C.D Odone D T., Engleman E.P & Splitter S.D : Radiology 80 837 1963
20 Report from th sub-c mmittee on diagnostic criteria for systemic lupus erythematosus. In Bennett, P.H. & Wood, P H.N., eds.: Population Studies of the Rheumatic Diseases, Proceedings of the third international symposium New York, 1966 pp 287–289 International Congress Series No. 148 Excerpta Medica Foundation, Amster dam 1968.
21 Ropes, M.W Medicine (Baltimore) 43 : 387 1964.
22. Siegal, M., Lee, S.L., Widelock, D Reilly E.B Wise, G.J Zingale S.B & Fuerst, H.T J chron. Dis. 15 131 1962.
23 Siegal, M. & Lee S.L. In Bennett, P.H. & Wood, P.H.N eds. Population Studies f th Rheumatic Diseases, Proceedings of the third international symposium New York, 1966 pp 245– 57 International Congress Series No 148 Excerpta Medica Founda-ti n, Amsterdam 1968
24 Statistical Yearbook of N rway Central Bureau of Statistics of Norway Oslo 1959–1963
25 Stevens, M.B Abbey H. & Shulman, L.E. New Engl. J Med. 268 976 1963
26. Talbott, J.H. In Dubois, E.L., ed.. Lupus Erythematosus, pp 1–9 McGraw Hill Book Company New York 1966.
27 Weir D.M., Holborow E.J & Johnson, G.D Brit. med. J 1 933 1961
28. Zinkham W.H. & Conley C.L. Bull. Johns Hopk. Hosp. 98 102 1956.

II Development of an indirect LE factor test

ROLF A. LARSEN & ARNE BØYUM
Section for Immunology Institute f Experimental Medical Research,
University f Oslo Ullevaal Hospital, Oslo and Norwegian Defence
Establishment, Division for Toxicology Kjeller Norway

ABSTRACT
An indirect LE factor technique, including simple method for separation of leucocytes, is described. In the test standardized amount f serum reacts with constant amount of damaged nuclei serving as antigen, and fagocytosis is secured by addition of constant amount of freshly-prepared leucocytes. It is possible to titrate LE sera. The titration of LE sera gives regular succession of different phenomena associated with LE cell. This gradual change in the morphology has been described. Granulocyte- and lymphocyte-specific LE factors are demonstrated.

The LE cell phen menon was originally described in bone marrow (11) later in venous blood (12 23) and by ddition of LE plasma to bone marrow (13) Investigations int this phenomenon, with elucidation of its mechanism have contributed greatly t our understanding of the immunological aberrations in systemic lupus erythematosus (SLE)

Three materials interact in the development of this phenomenon- the LE factor occuring in the patient plasma, interacts with constituents in damaged nuclei. This leads to morphological changes of the nuclear substance with f rmation of LE bodies. Finally these are fagocytized by living granulocytes with f rmation of LE cells.

The LE cell phenomenon may be demonstrated by direct techniques when serum and leucocytes originat from the patient (for references, see 5) In indirect techniques, serum from the patient interacts with leucocytes from another source.

Th initial phase f th LE phenomenon, i.e. th reaction between the LE factor and nuclear substance does not need complement (1 17) Complement is essential however for optimal fagocytosis, and formation f true LE cells.

The LE ell phenomenon has varied and rather complex morphology and the components have been described by different names. Detailed information is available in reference 5 The LE factor behaves as typical antibody directed against antigens present in nucleoprotein, requiring both DNA and histone for complete activity (15)

The purpose of the present investigation was to develop technique for evaluation of the LE cell phenomenon in material of patients with systemic lupus erythematosus and their relatives. This required the use f sera with a possibility of determining the titre of the activity and parallel tests with known and serial dilutions of positive control sera. A onstant source of antigen was also required. The present paper describes an indirect technique for demonstration f the LE factor which fulfills these criteria.

MATERIALS AND GENERAL TECHNIQUES

Sera. Sera were obtained from patients with systemic lupus erythematosus and their relatives, as described elsewhere (19 20) The sera were stored at 22 C in small aliquots and thawed once immediately prior to testing.

Separation of leucocytes: The technique and theory for separation of leucocytes has previously been described in detail (3) The separation f isolated granulocytes and isolated lymphocytes has also been described elsewhere (4)

Isopaqu (natril-N-methyl-3,5-diacetamido-2 4,6-triojodbenzoas) 75 per cent was provided by Nyegaard and Co., Oslo Norway and dextran 500 or dextran 250 were obtained from Pharmacia, Uppsala, Sweden. A stock solution containing 10 volumes Isopaque 33.9 per cent and 20 volumes dextran 250 was prepared, this solution has a density of 1 08 g per ml and is stored at 4 C. Two or three ml of the Isopaque-dextran stock solution were dded to tubes of 100 x 16 mm and a similar amount of newly-drawn blood containing 10 IU heparin per ml was layered over the stock solution. The erythrocytes formed clumps which sedimented to the bottom of the tube, leaving a plasma layer containing 6–8000 leucocytes per mm^3

The separated leucocytes were used in the test both as a source of antigen and as fagocytes as foll ws

Antigen. 1) Preparation Leucocytes were damaget by freezing and thawing. 2) Standardization: Heparinized blood from healthy group 0 donors was used for preparation of leucocyte-rich plasma which was frozen in small aliquots after counting the leucocytes. They were thawed immediately before use and diluted by heparinized pooled normal serum containing 20 IU heparin per ml. The amount of antigen in the test mixture was kept constant by varying the amount of normal serum. This is required so as to have constant conditions in the test, as described below The antigen should be used immediately after thawing. 3) Storage Storage of antigen in th frozen state should not be for more than 3 weeks (vide infra)

Fagocytes. Leucocytes in the plasma layer were also used as living ells for f gocytosis and thus formation of LE ells. The cells were used immediately after separation, but survived stor age overnight at 4 C. The number of fagocytosing cells was standardized to obtain constant conditions during the test.

Performanc f the t st Half millilitre test serum 0.2 ml antigen (ontaining 5×10^5 nuclei) and 0.3 ml fresh leucocyte-rich plasma (containing ..3 10 leucocyt) were dded to a small test t be (80 mm 10 mm) The tube was stoppered, haken to nsure omplete mixing of the contents and kept 37 C for 90 minutes. The tubes were gently sh ken 2–3 times during this in ba ion pe od Aft incubation, the tubes wer unf ged f 4 minutes at 600 g at 20 C. The supernatant was removed and the thin cellular layer on the bottom of the tube harvested by a thin, dry Pasteur pipette. Two drops were placed on glass slides and smeared between two slides to obtain a concentrated and single layer of intact cells (10) Complete removal of the supernatant fluid prior to harvesting the cells is important. Careful preparation of the smear is also an important requirement for optimal evaluation of the LE cell phenomenon.

Positive and negative controls were always run in parallel, negative controls being selected normal sera and positive controls sets of dilutions of known positive sera.

Staining. The slides were first incubated with methanol for 15–30 minutes, and then with May-Grünewald stain (A.G Merck, Darmstadt, Germany 1 volume and 1 volume 0.2 M phosphate buffer of pH 6 8) for 15 minutes. Final incubation was with Giemsa stain (A.G. Merck, Darmstadt, Germany 1 volume plus 9 volumes of the phosphat buffer) for 5 minutes. The slides were then washed twice in the phosphate buffer

RESULTS

In the test, a standardized amount of serum reacted with a constant amount of damaged nuclei serving as antigen, and fagocytosis was secured by addition of a constant amount of freshly-prepared living leucocytes. It is established that th size form and staining of the LE body is dependent on the amount of LE factor reacting with the nuclei (9) With the present technique, the nuclei were damaged by prior freezing and thawing This leads to a constant degree of damage to the nuclei, and a similar amount of LE factor was bound to each damaged nucleus. As a result, the morphology was virtually onstant throughout each preparation, which m de microscopic reading rapid and simple.

Morphology of the LE ell phenomenon in the described test

When LE factor was absent, the nuclei remained unchanged and were seen as nuclear mate rial forming l ng strands between the intact



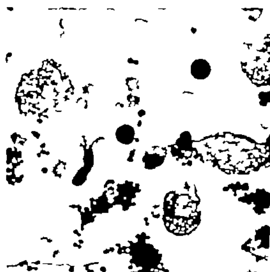


Fig. 1 *Negative LE phenomena.* A. The nuclei are unchanged and the nuclear material forms long strands between the intact living leucocytes. B. The nuclei are swollen, but the chromatine intact (ca. 420).

living leucocytes. This is illustrated in Fig 1A. Sometimes, dense small, irregularly-shaped nuclei were seen, and, more rarely swollen nuclei with intact chromatine (Fig 1B)

Large amounts of LE factor usually resulted in formati n of numerous LE ells throughout the preparation. The appearance of the preparation is an indication of the amount of LE factor present in th serum but better information is obtained through titration f the serum and determination of a titre which corresponds to the highest dilution of serum giving distinctly positive phenomenon.

The gradual change in the morphology of the LE cell phenomenon is illustrated in Fig by th findings using different dilutions f strongly positive LE serum R.M., obtained from female patient with systemic lupus erythematosus:

Fig 2A illustrates th morphology when the serum is diluted 1 4 Serum R.M. shows prozone phenomenon when used undiluted, as only LE bodies are formed. There are clusters f classical LE cells with multiple inclusions of variou sizes rosettes and LE bodies are rare. The inclusion bodies are seen in granulocytes. When round homogeneous LE bodies are present, they are small.

When the serum is dil ted 1 100 a shift from LE cells to rosettes is seen (Fig. 2B) The rosettes consist of leucocytes around LE bodies which are usually larger and less homogeneous than free LE bodies. Some of the inclusion bodies of LE cells are also less homogeneous.

Fig 2C shows forms of rosettes obtained when the serum is diluted 1 400 The LE bodies within the rosettes are usually disintegrated; these true rosettes are sometimes difficult to discriminate from false forms formed by leucocytes around other materials, usually platelets.

Fig. 2D shows LE bodies which are irregularly-shaped and less homogeneous. Som LE bodies are ttached to leucocytes and disinte grated at the sit f attachment. The conditions for complete rosette formation are not present, as th serum is dil ted 1 800

When serum R.M. is further dil ted, the shape of th LE bodies becomes even more irregular and less homogeneous. The titre is 1 3200 and orresponds to the highest dilution giving a distinct nuclear change. Finally unchanged nuclear material is seen between the intact living leucocytes. Parallel tests with known negative sera sustain these negative findings.

The different omponents f the LE cell phenomenon vary in morphology Fig. 3 shows LE cells with a coarser structure of the inclu

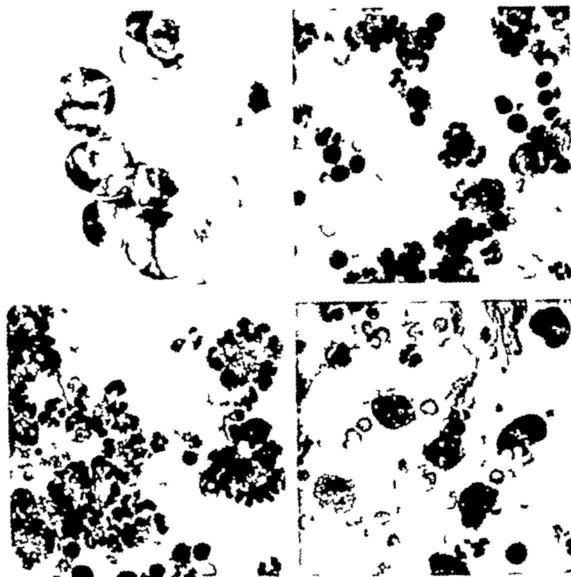


Fig. 2. *Positive LE phenomena.* The various morphological phenomena appear with increasing dilution of a strong LE serum, obtained from female SLE patient R.H. A. *N* *merous* *lassical LE cells.* Note the clusters of multiple inclusions of various size. Serum 1 4 (ca. x 900). B. *LE* *ells and rosettes.* Note the shift from LE cells only to LE cells and rosettes. Rosettes consist of leucocytes around LE bodies. Serum 1 100 (ca. 420). C. *R* *settes.* LE bodies within the osettes are disintegrated. True rosettes are sometimes difficult to discriminate from *false forms formed* by leucocytes around other materials, usually platelets. Serum 1:400 (ca. 420). D. *LE bodies.* Note that the Le bodies are larger, more irregularlyshaped and less homogeneous than the LE bodies shown in Fig. 4A. Some LE bodies are attached to leucocytes, but complete rosette formation is absent. Serum 1 800 (ca. 420).

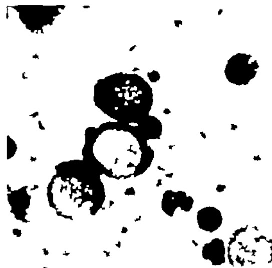
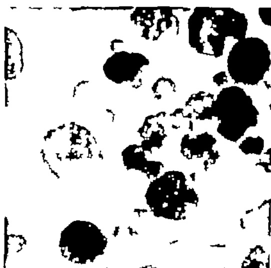


Fig. 3. *Atypical LE cells.* Note the coarse structure of the inclusion bodies. This strong LE serum M.G. was obtained from female SLE patient with uremia, the LE bodies exhibit the same appearance (ca. 900).



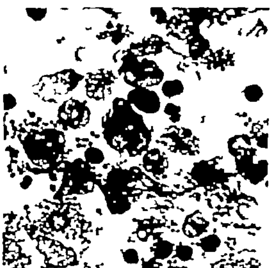


Fig. 4. *Types f LE bodies.* A. *Classical round homogeneous LE bodies.* Note the shape and size of the LE bodies. This type is observed when larger amounts of LE factor are present (ca. 900). B. *«Lacy» LE bodies.* These are usually found when small amounts of LE factor are present. The LE bodies are different from those shown in Fig. 2D, which illustrate distinctly positive LE phenomenon, and from the swollen nuclei seen in Fig. 2B, which illustrate negative LE phenomenon (ca. 420).

sion bodies than usually seen. This was b-tained with serum M.G diluted 1 2 from female SLE patient with uremia, th LE bodies exhibit the same appearance even when the serum is further diluted. The titre is 1 64. The patient had similar titre early in active disease but the morphology of the LE cell phenomenon was at that time like that illustrated in Fig. 2A–D

Fig. 4A shows *classical round homogeneous* LE bodies; sometimes the LE bodies melt together and form large irregular shapes. LE sera giving such large bodies rarely exhibit LE cells.

Fig 4B shows LE bodies of lacy reticular appearance which are usually seen when the LE phenomenon is weakly positive Sometimes the lacy round LE bodies form larger bodies.

The influence f varying the conditions for the test.

Selection f th amount of serum damaged nuclei and ther conditions for the test were based upon systemic experiments, the conditions for th test being varied one t time. Some of this information will now be de scribed

Table I. Titres of various morphological LE phenomena when different amounts of nuclei (antigen) were incubated with a constant amount of serum.

Leucocyte nuclei per ml LE serum	Reciprocal f titre		
	LE bodies	Rosettes	LE cells
2.8×10^6	400	200	200
$1\ 4 \times 10^6$	1600	800	400
$0\ 7 \times 10^6$	3200	1600	800
0.2×10^6	3200	–	3200

Table I shows the findings when one serum (R.M.) was incubated with varying amounts of damaged leucocytes. It is evident that th titre decreased for formation of LE bodies, rosettes and complete LE cells when the amount of nucleir was increased relative to th amount of serum The amount of damaged nuclei used in the test was selected as the amount providing optimal sensitivity of th test. Under special conditions where increased sensitivity is desirable, the amount of serum relative to damaged nuclei can be increased over that specified.

Table II illustrates that th properties of the antigen changed during storage at 22 C. This effect was not due to development of anticomplementary activity since the other morphological features of the LE phenomen n were similarly affected. Antigen stored for less than 3 weeks was theref re used in the test.

The morphological appearance also depended on the number of living leucocytes relative t the am unt of damaged nuclei. Table III shows the results of some experiments where th amount of serum was kept constant, while the numbers f living leucocytes and nuclei were varied. When th amount of damaged

Table II. The effect f storage of antigen at 22 C on the LE cell phenomenon induced by a strong LE serum R.M.

Storage of antigen in weeks	Reciprocal titre of LE cells
0	800
2	800
4	200
8	200
10	50
14	25
16	5
20	5
24	<1

nuclei was decreased relative to the numbers of leucocytes, it lead to formation of more com plete LE cells relative to LE bodies. When the numbers of living leucocytes were also lowered, a decreasing amount of damaged nuclei lead to formation of even more complete LE cells in relation to LE bodies.

One aspect of the importance of selection of antigen donor is shown in Table IV It is evident that the formation of LE bodies, rosettes and complete LE cells caried in different LE sera when the amount f nuclei was decreased relative to the amount of serum.

Isolated granulocytes and isolated lymphocytes were used for preparation of antigen, and then tested with series of known positive sera. Table V shows that the titres of 5 sera varied considerably when tested with granulocytes and lymphocytes respectively Serum E.L. showed a higher titre against granulocytes than lympho-

Table III The ratio of LE cells/LE bodies when nuclei and facocytes are gradually decreased

Leucocyte nuclei per ml LE serum	Living leucocytes per ml LE serum			
	$5\ \ 10^6$	$2.5\ \ 10^6$	1.25×10^6	$0\ 6\ \ 10^6$
$3\ 6\ \times 10$	$<0\ 01$	0.3	0.5	1 0
$1\ 8\ \times 10^6$	0 01	0.5	2.0	3 0
$0\ 9\ \times 10^6$	0 02	0.5	2.0	>10
$0.45\ \ 10^6$	0 04	0 5	2 0	>10
$0\ 22\ \ 10^6$	0 10	0 5	–	>4

Table IV Various morphological LE phenomena in strong LE sera when different amounts of nuclei (antigen) were incubated with constant amount of undiluted serum

Leucocyte nuclei per ml LE serum	SLE patient G. H.	SLE patient A. L.	SLE patient A. J
2.8×10^6	LE bodies	LE bodies	LE bodies
1.4×10^6	LE bodies	Rosettes	LE bodies
0.7×10^6	Rosettes	LE cells	LE bodies
0.2×10^6	LE cells	LE cells	LE bodies

Table V The effect of using isolated granulocytes or isolated lymphocytes in the indirect LE factor test.

LE serum from	Reciprocal of titre with Nuclei of Granulocytes	Reciprocal of titre with Nuclei of Lymphocytes
E. L.	4	<1
S. K.	<1	4
N. O	8	8
K. H	4	16
M. H.	16	8

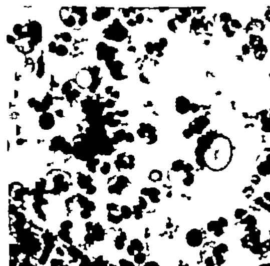




Fig. 5. Granulocyte-specific LE factor. A. Isolated granulocytes are used as antigen and tested with serum E.L. Note that LE cells are formed. B. Isolated lymphocytes are tested with serum E.L., but the nuclei are unchanged and are seen as strands (ca. 420).



Fig. 6. *Lymphocyte-specific LE factor.* Isolated lymphocytes are used as antigen and tested with serum S.K. Note the LE bodies. When isolated granulocyte nuclei are tested with serum S.K., the nuclei are seen as in Fig. 4B (ca. 420).

cytes, whereas sera S.K. and K.H. showed higher titres against lymphocytes than against granulocytes.

Fig. 5A shows LE cells formed when isolated granulocytes were used as antigen and tested with serum E.L. When a similar amount of isolated lymphocytes from the same donor was tested with serum E.L., the nuclei remained unchanged, as shown in Fig. 5B Serum E.L. also showed a positive antinuclear factor reac tion only when granulocytes were used as antigen in the immunofluorescent technique. The granulocyte-specific ANFs of serum E.L. were kindly demonstrated by Dr Elling, Auto-Immune Laboratory Statens Seruminstitut, Copenhagen Danmark.

Fig. 6 shows LE bodies when lymphocytes were used as antigen and tested with serum S.K. Th nuclei of granulocytes were unaltered when tested with serum S.K.

DISCUSSION

The LE factor was originally defined as the factor that induced the morphologically typical LE cell phenomenon (14) Adequate criteria have been described for tru LE cells (21) but other related morphological phenomena may be present alone or together with typical LE cells. The LE cell tests often provid conditions insufficient for optimal fagocytosis (6) and the significance of the other morphological patterns has not been established.

A quantitative assessment of the LE factor has previously been regarded as very difficult, particularly since the morphological phenomena may differ in various fields of the preparations (16) In th presently described experiments th leucocytes wer damaged by freezing and thawing This procedure was originally introduc d by *German & H ber* (8) and used by *Lachman* in his two-stage indirect LE cell test (16) In the present technique ther is onstant degree of damage f th nuclei and a similar amount of LE fact is bound to each damaged nucleus Th morphology is therefore onstant thr ghout ach p epar ti and t is possible t determin the t f LE fac or activity by the use f stan our f

antigen. The assessment of the LE factor in this indirect test is largely based on the effect of the first step of the LE phenomenon, namely the interaction between damaged nuclei and the LE factor The same principle is applied in the «loose body test» (22) When the other conditions are kept constant, the various morphological phenomena appear regularly with increasing dilution of strong LE sera (9) Strong SLE sera sometimes show prozone phenomena, as only LE bodies are formed, but undiluted strong LE sera usually exhibit numerous inclu sion bodies. The best conditions for fagocytosis were achieved when the tubes were incubated at 37 C for 90 minutes.

Antinuclear factors react with different nuclear antigens (2 18 24) Granulocyte and lymphocyte-specific ANFs have been described (7) This is probably a parallel to the present demonstration of granulocyte-specific and lymphocyte-specific LE factors. Cell specific LE factors are noted when the standard antigen which consists mainly of granulocytes is employed, but have to be sustained in additional tests with isolated granulocytes and isolated lymphocytes. Most frequently granulocyte specific LE factors are found.

The complex morphology of the LE phenomenon probably expresses qualitative and quantitative differences in the reaction between several LE factors and various antigenic structures in nuclei, and not interaction of an IgG antibody of limited specificity with nucleoprotein.

REFERENCES

1 Aisenberg, A.C. J clin. Invest. 38 325 1959
2 Beck, J.S. Lancet 1 1203 1061
3 Bøyum A. Scand. J clin. Lab. Invest. Suppl. 97 9 1968
4 Bøyum A. Scand. J clin. Lab Invest. Suppl. 97 77 1968.
5 Dubois, E.L. In Dubois, E.L., ed. Lupus Erythematosus pp 302–331 McGraw Hill Book Company New York 1966.
6 Engel, H –J Blut 17 95 1968
Faber V & Elling, P Acta med. scand. 179 5 1966

8 German, J.L. & Huber M Proc. Soc. exp Biol. (N Y) 97:2 1 1958.
9 Golden, H.E. & McDuffie, F.C.: Ann. intern. Med. 67: 780 1967
10. Hammer G., Scand. J clin. Lab. Invest. 1 61 1964.
11 Hargraves, M.M., Richmond, H. & Mort n, R.. Proc. Mayo Clin. 23 25 1948
12 Hargraves, M.M.: Proc. Mayo Clin. 24 234, 1949
13 Haserick, J.R. & Bortz, D W J invest. Derm. 13 47 1949
14 Haserick, J.R., Lewis, L.A. & Bortz, D W Amer J med. Sci. 219 660 1950.
15 Holman, H. & Deicher H.R. J clin. Invest. 38 2059 1959
16 Lachmann P.J Immunology 4: 142 1961
17 Lachmann P.J Immunology 4 153 1961
18 Lachmann, P.J & Kunkel, H.G : Lancet 2: 436, 1961
19 Larsen, R.A.: Acta med. scand. Suppl. 543 p 11 1972.
20 Larsen, R.A. Acta med. scand. Suppl. 543 p. 31 1972
21 Marmont, A., Fiuma, M.A. & Capponi, G.: Schweiz. med. Wschr 87 1253 1957
22 Soeren, F van Ann. heum. Dis. 20 281 1961
23 Sundberg R.D & Lick, N.B J invest. Derm 12 83 1949
24. Tan, E.M. J Lab. clin. Med. 70 800 1967

III Presence of LE factor in relatives and spouses

ROLF A. LARSEN
Section for Immunology Institute for
Experimental Medical Research
University of Oslo, Ulleval Hospital, Oslo 1 Norway

ABSTRACT
LE factor was determined in sera of 928 family members of 72 patients with SLE by an indirect LE factor test. The prevalence of LE factor was significantly increased in relatives compared with their spouses, female first degree relatives presenting the highest frequencies. The presence of LE factor in relatives was correlated t presence of rheumatic disease, but LE factor also occurred in apparently healthy relatives. The frequency f LE factor was independent of size of sibships and families and residence of the relatives.

The LE cell phenomenon is due t antinuclear antibodies reacting with nuclei of damaged cells (for references, see 18) This initially leads t morphological changes of the nuclei with formation of LE bodies. Subsequently the LE bodies are engulfed by living whit blood cells to form LE cells. LE bodies, rosettes, and LE cells occur in LE cell preparations, but the significance of th various morphological le ments is still disputed. LE ells are found in systemic lupus erythematosus (SLE) and re lated disorders (16)

Several to-antibodies, including antinuclear factors (ANF) occur with increased frequency in families of patients with SLE (for references, see 8) A test f LE factor has rarely been included in such studies (25 33 34) mainly for technical reasons. As part of an extensive study of clinical and serological aber rations in relatives f patients with SLE th results of an indirect LE factor test are pre sented in this paper A marked familial aggregation of LE factor was found, and LE factor was demonstrated in healthy individuals.

MATERIALS AND METHODS

SLE probands. The criteria for selection of the 72 probands with SLE have been publish d elsewhere (19) The probands represented all individuals considered to be definit SLE cases in defined area from central eastern Norway during th years 1959–1965

Th family material. Initially the SLE patients their closest available family members (parents, spouse or children) were asked if they would participat in the present study and inform and persuade the other family members. Subsequently visits to the homes were arranged in cooperation with the initially contacted persons who accompanied th author on the visits. All available family members, irrespective f residence in Norway or adjacent parts of Sweden, completed questionnaire were clinically examined, and while in the fasting stat provided 50 ml blood sample. First degree relatives (children, siblings and parents) second degree relatives (siblings of parents) and their spouses were primarily included in the investigation. Th spouses of the SLE probands served as first degree controls together with th spouses of the siblings. »Others» relatives, mainly grandparents and grandchildren, were also included, when vailable. The pedigrees were submitted t and confirmed by the other family members, and additional data collected from their physicians files and hospital records. The data from the questionnaires, physicians nd hospital records, clinical and serological examinations (about 700,000 in all) were punched on data cards f electronic data processing

Table I Survey of relatives of the 72 SLE probands and their spouses.

Group	Sex	Tested and clinically examined		Refused to cooperate		Completion rates (%)		Not available but data present		Unknown,* * data not present	
		Relatives	Spouses	Relatives	Spouses	Relatives	Spouses	Relatives	Spouses	Relatives	Spouses
First degree	female	191	78	3	15	98.5	83.9	44		40	23
	male	178	109	9	11	95.2	90.8	41		53	38
	both	369	187	12	26	96.8	87.8	85		93	61
Second degree	female	105	56	9	12	92.1	82.4	29	1	166	161
	male	97	53	15	5	86.6	91 4	38		172	175
	both	202	109	24	17	89.3	86.5	67	1	338	336
First and second degree	female	296	134	12	27	96 1	83.2	73	1	206	184
	male	275	162	24	16	91.9	91.0	79		225	203
Other	both	44	17					3			
T tal	both	615	313	36	43	94.5	87.9	155	1	431	387

* Abroad or deceased
Most of the relatives had died shortly after birth, and the spouses were usually married to deceased relatives.

A survey of groups of relatives (of the 72 probands with SLE) and their spouses is given in T ble I.

Approximately 63 per cent of first and second degree relatives were included in the present LE factor st dy but available information was present from an additional 155 relatives either abroad o deceased. Thus, data were present from 83 per cent of first degree relatives. Most of the relatives with no available information had died shortly after birth. The completion rates in the groups of relatives varied from 86 6 per cent to 98.5 per cent. The spouses f the SLE probands are included in Table I. The ompletion rates in groups of spouses varied from 82 4 to 91 4 per cent. These completio rates are in greement with the claims for famdy studies (23) Unknown spouses were usually marned t deceased relatives. The ge and sex distributi n of th relatives and spo ses, all tested and clinically examined, is shown m tables II and III.

The ratio between relatives and spo se was 2 1

Statistical methods. The data were processed in a CD 3300 computer at the University of Oslo Norway The age distribution usually differed in the various populations. ANF positi v ty is regarded as being age-dependent (2 5 32) χ^2 calculations were therefore performed separately for each age group and the total group (obtained by summing up the individuals in the various age groups) but significant differences (p-values below 0 05) between total groups were usually not taken into account. When the task was to combine evidence for the three age groups (sets of 2 x 2 tables) Mantel-Haenszel's method (M–H) was employed (28). A continuity correction is used (9 *8)

All p-values of significant level (< 0 05) are given in the tables. However when the p-values in specified groups seem to contribute to significant differences when groups are summarized, non-significant p-values are also indicated. In some instances, non-significant p-values are also given.

Indirect LE f ctor t st The technique has been described in detail elsewhere (18). The

Table II. Age and sex distribution of groups f relatives for the 72 SLE probands.

Group	Sex	Age distribution (yrs)			
		<40	40 – 59	≥60	T tal
Offspring	female	34 (94 4 *)	2 (5 6)	0	36 (100.0)
	male	39 (88 6)	5 (11.4)	0	44 (100.0)
Siblings	female	34 (28.3)	62 (51 7)	24 (20 0)	120 (100 0)
	male	22 (21.5)	60 (58 9)	20 (19.6)	102 (100 0)
Parents	female	0	8 (22.9)	27 (77.1)	35 (100.0)
	male	0	7 (21.9)	25 (78 1)	32 (100.0)
Total first degree	female	68 (35.6)	72 (37 7)	51 (26 7)	191 (100 0)
	male	61 (34.2)	72 (40 5)	45 (25.2)	178 (100 0)
	both	129 (34 9)	144 (39.0)	96 (26 0)	369 (100 0)
Sib of mother	female	1 (1.5)	23 (33.8)	44 (64 7)	68 (100 0)
	male	3 (5.5)	23 (41.8)	29 (52 7)	55 (100.0)
Sib of father	female	0	11 (29 7)	26 (70.3)	37 (100.0)
	male	1 (2.4)	15 (35 7)	26 (61.9)	42 (100.0)
Total second degree	female	1 (1.0)	34 (32.4)	70 (66 6)	105 (100 0)
	male	4 (4 1)	38 (39.2)	55 (56.7)	97 (100.0)
	both	5 (2.5)	72 (35 6)	125 (61 9)	202 (100 0)
First and second degree	female	69 (23.3)	106 (35.8)	121 (40 9)	296 (100 0)
	male	65 (23 7)	110 (40.0)	100 (36.3)	275 (100 0)
Other	both	24 (54.5)	9 (20.5)	11 (25.0)	44 (100.0)
Total	both	158 (25 7)	225 (36 6)	232 (37 7)	615 (100.0)

No. f individuals in the specified age group
** Percentage individuals in the specified age group

sera were coded, stored in small aliquots at 22 C, thawed once before use recorded by technical assistant, and the tests read by double blind technique. A standardized antigen from single healthy blood donor was employed, the antigen was stored for less than thre weeks bef re use (18). Positive sera were tested in twof ld serial dilutions, and weakly positive sera were retested. All tests were perf rmed in duplicate. Positive and negative controls were included every day. The criteria f positive tests and determination f titres have been described in detail elsewhere (18). Th last dilution which induced morphological changes of the nuclei making them distinctly differet from th negative (normal) control determined th titre. Sera with titre of 1/1 and higher in the indirect LE factor test were considered as positive

Table III Age and sex distribution of controls (spouses of relatives of the 72 SLE probands).

Group	Sex	Age distribution (yrs) <40	40 – 59	>60	T tal
Offspring	female	7 (77.8)	2 (22.2)	0	9 (100.0)
	male	4 (40.0)	6 (60.0)	0	10 (100.0)
Siblings	female	22 (31.9)	39 (56.5)	8 (11.6)	69 (100.0)
	male	19 (19.2)	65 (65 7)	15 (15.2)	99 (100.0)
Total first degree	female	29 (37.2)	41 (52.6)	8 (10.2)	78 (100.0)
	male	23 (21.1)	71 (65 1)	15 (13 7)	109 (100 0)
	both	52 (27.8)	112 (59.9)	23 (12.3)	187 (100.0)
Sib. of mother	female	2 (6.7)	16 (53.3)	12 (40.0)	30 (100 0)
	male	0	10 (25.0)	30 (75.0)	40 (100.0)
Sib of father	female	2 (7 7)	16 (61.5)	8 (30.8)	26 (100.0)
	male	0	5 (38.5)	8 (61.5)	13 (100.0)
Total second degree	female	4 (7 1)	32 (57 1)	20 (35.8)	56 (100.0)
	male	0	15 (28.3)	38 (71 7)	53 (100.0)
	both	4 (3 7)	47 (43 1)	58 (53.2)	109 (100.0)
First and second degree	female	33 (24 6)	73 (54.5)	28 (20.9)	134 (100.0)
	male	23 (14.2)	86 (53.1)	53 (32.7)	162 (100.0)
Other	both	8 (47 1)	5 (29 4)	4 (23.5)	17 (100.0)
T tal	both	64 (20 4)	164 (52.4)	85 (27.2)	313 (100.0)

The figures are recorded as in Table II

RESULTS

A. The prevalence of LE factor in relatives and spouses.

The prevalence of LE f ctor in th relatives is shown in Table IV

LE factor was present in high percentage (26.7) of female first degree relatives, the sex difference in frequency of LE fact was less pronounced in second degree relatives. Increased frequency f LE fact by age wa very irregular Younger ffspri g, middl aged siblings and elderly p ents show d th highest figures.

Th prevalence of LE factor in the spouses is presented in Table V

The figures were lower than f the relatives, and there was no sex or age difference. LE factor occurred three times more frequently in female relatives than in female spouses. The results of some of th statistical computations of the differences between relatives and spouses are shown in Table VI.

Statistically significant differences were f und between female relatives and spouses in the highest age groups, mainly between middle-aged female groups. Significant differences were not bserved between male relatives and

Table IV *Indirect LE factor test:* Frequency of positive tests in groups of relatives of 72 SLE probands.

Group	Sex	Age distribution (yrs) <40	40 – 59	>60	Total
Offspring	female	8 (23.5**)	0		8 (22.2)
	male	1 (2.5)	1 (20 0)		2 (4.5)
Siblings	female	4 (11.7)	21 (33.8)	4 (16 6)	29 (24 1)
	male	0	5 (8.3)	3 (15 0)	8 (7.8)
Parents	female		2 (25.0)	12 (44 4)	14 (40.0)
	male		1 (14.2)	3 (12.0)	4 (12.5)
First degree	female	12 (17.6)	23 (31.9)	16 (31.3)	51 (26 7)
	male	1 (1.6)	7 (9 7)	6 (13.3)	14 (7 8)
	both	13 (10 0)	30 (20 8)	22 (22.9)	65 (17.6)
Sib f mother	female	0	4 (17.3)	9 (20 4)	13 (19 1)
	male	0	2 (8 6)	6 (20.6)	8 (14.5)
Sib of father	female		0	7 (26.9)	7 (18.9)
	male	0	2 (13.3)	4 (15.3)	6 (14.2)
Second degree	female	0	4 (11 7)	16 (22 8)	20 (19 0)
	male	0	4 (10 5)	10 (18.1)	14 (14 4)
	both	0	8 (11 1)	26 (20.8)	34 (16.8)
First and second degree	female	12 (17 3)	27 (25 4)	32 (26.4)	71 (23 9)
	male	1 (1 5)	11 (10.0)	16 (16 0)	28 (10 1)
Other	both	4 (16.6)	0	2 (18 1)	6 (13.6)
Total	both	17 (10 7)	38 (16 8)	50 (21.5)	105 (17.0)

No of positive in the group

** Percentage of positive in the group

Table V *Indirect LE factor test* Frequency of positive tests in groups f spouses of relatives of 72 SLE probands.

Group	Sex	Age distribution (yrs) <40	40 – 59	>60	Total
Off-spring	female	0	0		0
	male	0	1 (16.6)		1 (10 0)
Siblings	female	2 (9 0)	4 (10.2)	0	6 (8.6)
	male	1 (5.2)	2 (3 0)	2 (13.3)	5 (5 0)
First degree	female	2 (6.8)	4 (9 7)	0	6 (7.6)
	male	1 (4.3)	3 (4.2)	2 (13.3)	6 (5.5)
	both	3 (5 7)	7 (6.2)	2 (8.6)	12 (6 4)
Sib of mother	female	0	1 (6.2)	0	1 (3.3)
	male		0	2 (6.6)	2 (5.0)
Sib of father	female	1 (50.0)	0	0	1 (3 8)
	male		0	0	0
Second degree	female	1 (25.0)	1 (3 1)	0	2 (3.5)
	male		0	2 (5.2)	2 (3 7)
	both	1 (25.0)	1 (2.1)	2 (3 4)	4 (3 6)
First and second degree	female	3 (9.0)	5 (6.8)	0	8 (5.9)
	male	1 (4.3)	3 (3 4)	4 (7.5)	8 (4.9)
Other	both	2 (25.0)	0	0	2 (11 7)
T tal	both	6 (9.3)	8 (4.8)	4 (4 7)	18 (5 7)

The figures are recorded as in Table IV

spouses, but th degree of significance was higher when both sexes were combined. The degree of significance was highest when all age groups were combined and used in the calculations. Th difference in occurrence of LE factor in the total populations of relatives and spouses was highly significant.

B. The prevalence f LE f t in heumatic and non-rheumatic relative and spouses.

Th f llowing variables wer considered pre sence of at least tw of ARA criteria for rheumatoid arthritis (RA) (7) effusions in small (toes and fingers) and large (wrists, elbows, shoulders, knees and ankles) joints, juvenile rheumatoid arthritis, ankylosing spondylitis, Reiter syndrome bursitis, diffuse muscular pains, tenosynovitis, rheumatic fever scleroderma, dermatomyositis, or gout. A non-rheumatic member lacked these variables; a rheumatic member had at least ne of the variables.

Relatives. There was a difference in the prevalence of LE factor in rheumatic and non-rheumatic relatives In first degree relatives, LE factor occurred twice as frequently in heumatic as in n n-rheumatic individuals.

Table VI. *Indirect LE factor test*. Statistical analysis of the differences between relatives (of 72 SLE probands) and their spouses. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups

		Age distribution (yrs)			All age groups
		<40	40 – 59	>60	combined
Group	Sex	df 1	df 1	df 1	M-H. two-tailed
Siblings	female		0.02		0.0104
First degree	female		0 02		0.0012
	both		0.002		0 0006
Second degree	female				0.025
	male				0.052
	both			0 005	0 002
First and	female		0 003	0 005	<0.0001
second degree	male				0 0854
Total	both		0 0005	0 0008	<0.0001

Rheumatic, male second degree relatives showed high prevalence (26.5 per cent) f LE factor whil in non-rheumatic male second degree relatives LE fact was almost absent. Rheumatic female first degree relatives showed no increased frequency of LE factor by age, while non-rheumatic female first degree rela tives had an age-dependent frequency

The results of some of the statistical calculations concerning the difference in prevalence of LE factor between rheumatic and non-rheumatic relatives are shown in Table VII.

Statistically significant differences between rheumatic and non-rheumatic relatives were found in male second degree relatives (mainly due to aged males) middle-aged first degree relatives (mainly due to males) aged second degree relatives and young relatives f the total populations (mainly due to females) When all age groups were combined and used in the calculations, statistically significant differences were also present in the same groups.

Spouses: Rheumatic spouses generally showed higher frequency of LE factor than non-rheumatic spouses, but the differences were small.

Rheumatic relatives and spouses:

The statistical calculations of some of the differences in prevalence of LE factor between rheumatic relatives and spouses are given in Table VIII.

Statistically significant differences were largely found between rheumatic relatives and spouses for th same groups as those recorded in Table VI, but the degree f significance was lower (cf Tables VI and VIII) The difference of LE factor between rheumatic male first and second degree relatives and spouses was more distinctly present, but still not statistically significant.

N n-rheumatic relatives and spouses still differed significantly in frequency of LE factor

C. LE factor and size of sibships and families

Two hundred sibships with 571 individuals occurred in first and second degree relatives. The sibship size varied from one to ten when SLE probands were not included. Totally LE factor was found in 34.5 per cent of the sibships once in 23 per cent, twice in 8.5 per cent, three times in 2.5 per cent and four times

Table VII. *Indirect LE factor test* Statistical analysis of the differences between relatives (of 72 SLE probands) with and without rheumatic disease Methods: χ^2 calculations and Mantel-Haenszel's method (M-H). Degrees of freedom (df) are indicated The figures show the p-values in different age and sex groups.

Group	Se	Age distribution (yrs) <40 df 1	40 – 59 df 1	≥60 df 1	All age groups combined M-H. two-tailed
First degree	female		0.34	0 76	0 09
	male		0 17		
	both		0.0456	0 72	0 0046
Second degree	female			0 43	
	male			0 003	0.003
	both			0.006	0.012
First and second degree	female		0.51	0 48	0.063
	male		0 1	0 16	0.025
Total	both	0 02	0.09	0 106	0 00088

Table VIII *Indirect LE factor test* Statistical analysis f the differences between relatives (of 72 SLE probands) and their spouses, all presenting evidence of rheumatic disease Methods: χ^2 calculations and Mantel-Haenszel's method (M-H) Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	Age distribution (yrs) <40 df 1	40 – 59 df 1	≥60 df 1	All age groups combined M-H. two-tailed
First degree	female		0 17		0.12
	male				
	both		0.024		0.028
First and second degree	female		0 04	0.03	0 003
	male		0 17	0.31	0.056
Total	both		0.012	0 008	0.0002

In a ingl albship In a sibship of three all members presented LE factor

Th family siz varied from o e t eighteen when the SLE proba ds were not included. LE fact was present in two-thirds of th SLE families one in 7 7 per ent twice in 19 5 per ent three times in 1 .5 per cent, four times in 4.. per cent nd fiv mes in 1 4 per ent I family of si teen seven members presented LE fact r

Ther was no apparent increase of LE factor by sibship or family size and the aggregation of LE fa tor in relatives was not d e to an accumulatio of the facto in a minority f ibship f milies. Th distribution of LE f ct in sibships and families was calculated

and f und consistent with the Poisson distribution.

D. LE facto and the residence of relatives

One-fifth of the relatives lived outside th defined area in central eastern Norway in which the SLE probands were diagnosed (19) The prevalence f LE factor did not differ in relatives and controls living outside or inside this area.

E. Genetic basis for LE facto

A study of the genetic basis of the occurrence of an antibody is difficult, as the presence of an antibody requires both the gen(s) and an adequat stimulus, and the stimulus for the induction of LE factor is unknown.

The offspring of parents without LE factor often exhibited the factor It thus appears unlikely that the ccurrence of LE factor is due to singl dominant gene.

A method for checking the agreement of the observed segregation of a character in family data, and th expected segregations calculated n the basis of Mendelian ratios – i.e., for th simple case of two alleles, one recessive to the other – has been evolved by Smith (26 27) Smith method was used f LE factor as character in the present family material, but th results of the tests for agreement between observed and expected numbers of (i) recessives (with negative LE factor) and of (ii) affected (with positive LE factor) make it unlikely that the occurrence of LE factor is due to monofactorial dominant or recessive inheritance

As previously stated, the familial aggregation of LE factor was n t related to sibship or family size, nor was it d to accumulation f LE factor in mnority of sibships. A high prevalenc of condition (here LE factor) both in first and second degree relatives is suggestive of a *multifactorial (genetic)* influence and Falconer has developed method of assessing th likelihood of an individual developing the same multifactorially determined disease as relative (for references, see 6) If we assume that the prevalence of LE factor is similar in *spouses and in general* population, the heritability f the LE factor in first degree relatives was f und to be about 80 per cent, using Falconer s formulae. The prevalence of LE facto was 17 6 and 6 4 per cent in first degree relatives and spouses, respectively However th *prevalence of LE factor in second degree* relatives and spouses was even higher (16.8 per cent and 3 6 per cent, respectively) and as the coefficient of relationship for second degree relatives is 1/4 th approximate estimate f the heritability of LE factor from the present family sample becomes too high. This is probably partly due to the selection of SLE probands, as numerous LE cells is only one of the criteria upon which th diagnosis of SLE was based (19) Th frequency of LE factor is also age- and sex-dependent, which makes the calculation of heritability even more compli cated (11)

Tests f r penetrance were not performed.

DISCUSSION

LE factor was originally defined as factor that induced the morphologically typical LE cell phenomenon (15) here, LE factor denotes a factor giving any of the various morphological patterns (homogeneous and lacy round or amorphous LE bodies (globs) and rosettes) usually connected with true LE cells (1 15 30) The basis for this concept is given in detail in the paper describing the indirect LE factor test (18)

There are very few reports about the occur rence of LE factor in families f patients with SLE (25 33 34) This is usually due to lack f suitable techniques for demonstration of LE factor in population studies and to lack of adequat criteria for weakly positive LE factor tests.

Th prevalence of LE factor was determined in 615 relatives, and their 313 spouses, of 72 SLE probands from central eastern Norway by newly-developed, simple *sensitive and standardized* indirect LE factor test (18). There was significant increase f LE factor in relatives mpared with their spouses. All groups of relatives showed increased values, but the familial aggregation was most pronounced in female *subjects, the sex difference in frequency of LE* factor being very marked. Younger offspring,

middl -aged siblings and elderly parents showed the highest figures In first degree and elderly parents showed the highest figures; in first degree relatives, the frequency of LE factor was rather constant after forty years of age. In second degree relatives, the frequency of LE factor increased gradually by age. Familial accumulation of LE factor occurred both on the maternal and paternal sides and with similar frequency

Antinuclear factors (ANFs) detected by immunofluorescence using rat liver as source of nuclei, also showed familial aggregation in the present material (29) In some groups of relatives, the frequency of LE factor exceeded the frequency of ANFs. It is usually stated that sera giving positive LE cell phenomenon and no sign of ANFs by immunofluorescence occur very rarely and when present the phenomenon is said to be due to altered antigen in the former test (31) The higher frequency of LE factor in some groups of relatives in th present material may be due to different sources of nuclei in the two tests and to the interpretation of positive LE cell tests. Granulocyte-specific ANFs occur in sera and synovial fluids of patients with rheumatoid arhritis (10) Granulocyte-specific LE factor was demonstrated in sera of patients with systemic lupus erythema tosus (18) Nuclei of granulocytes are the main source of antigen in the indirect LE factor test (18) This favour demonstration of granulocyte-specific LE f ctor and might thus explain some of the cases with positive LE factor test and no ANFs detectable by the immunofluor escence test. Generally sera co taining LE factor give peripheral or homogeneous nuclear staining in immunofluorescence tests for ANFs (14) In the present study LE factor and homogeneous ANF were better correlat d in relatives than in spouses (29)

The prevalenc of LE facto in sera of relatives wa t corr lated to the size of sibships or f milies, as was the case for rheu matoid fact rs in other population studies (24) The number f members with LE f ctor increased gradually with in reasing family siz

Presence f LE f ct in ser of relatives wa partly relat d rheumatic dise se N -rheumatic relativ and spo se still differ d significantly in freq ency f LE f in ontrast to the findings w h g d ANF n th sam populations (29) Female rheumatic and non-rheumatic relatives do not differ while the male populations behave very differently

The cause of familial aggregation of LE factor and most other auto-antibodies is still obscure. The occurrence of rheumatoid factors (RFs) and ANFs in relatives of SLE probands has been interpreted as evidence for an inherited abnormality of th immune system (0) However a genetic study of rheumatoid arthritis (RA) and RF in Blackfeet and Pima Indians revealed no evidence that genetic fac tors played an important part in the etiology of RA and RF in these tribes (24) Both the studies in Indian tribes and in twins indicated that environmental factors are of prime im portance in the development of auto-antibodies, at least in apparently healthy individuals (3)

The presence of ANFs and LE factor in inbred strains of mice (22) probably parallels the observations made in families of patients with SLE. Although the mice are known to be infected with virus (21) experiments with hybrids stress the importance of genetic factors (4 12 17) for the occurrence of serological abnormalities and uto-antibodies.

The findings in the present family study do not fit any simple type of inheritance The increased, but irregular occurrence of LE factor in families of patients with SLE points to a multifactorial etiology of the LE factor where environmental factors probably play an im portant role Spouses of relatives were used as controls to reduce the importance of environmental factors. Although most family members had lived some distance from each other for many years, this did not affect the prevalence of LE f ctor It was difficult to achieve eal estimates of heritability of LE factor in the present family material when Falconer's meth od (6) including both environmental and genetic factors was used. The present material does not permit any conclusions as to the relative importance of genetic and environmental fac tors and the genetic mechanism involved in the aggregation of LE factor in families of patients with SLE, to solve th problem more information is needed on the mechanism of induction of LE factor

REFERENCES

1 Arterberry J.D., Drexler E. & Dubois, E.L.. J Amer med. Ass. 187 389 1964.

2 Beck, J.S Scot. med. J 8 373 1963

3 Boyle, J.A., Greig, W.R. & Buchanan, W W Wld med. J 14: 181 1967

4. Burnet, M. & Holmes, M.C. Nature (Lond.) 207 368 1965

5 Cammerata, R.J Rodnan, G.P & Fennell, R.H.: J Amer med. Ass. 199 455 1967

6. Clarke, C.A., Price Evans, D.A., Harris, R., McConnell, R.B. & Woodrow J C. Quart. J Med. 37: 1 1968

7 Committee of the American Rheumatism Associatio 1958 Revision f Diagnostic Criteria for Rheumat id Arthritis. B ll. rheum. Dis. 9 175 1958

8. Dubois, E.L. In Dubois, E.L., ed. Lupus Erythematosus, pp. 129–276 McGraw Hill Book Company New York 1966.

9 Dunn, O.J.. Basic Statistics: A Primer for the Biomedical Sciences, pp. 121–125 J hn Wiley & Sons, Inc., New York 1964

10 Elling, P., Graudal, H. & Faber V Ann. rheum. Dis. 27 225 1968

11 Falc ner D.S Ann. hum. Genet. 31 1 1967

12. Friou, G.J., Teague P.O & Myer L. Arthr and Rheum. 11 481 1968

13 Golden, H.E. & McDuffie F.S. Ann. intern. Med. 67 780 1967

14. Gonzales, E.N & Rothfield, N F New Engl. J Med. 274 1333 1966.

15 Haserick, J R., Lewis, L.A. & Bortz, D W Amer J med. Sci. 219 660 1950.

16 Hijmans, W., Doniach, D Roitt I.M. & Holborow E.J Brit. med. J 2 909 1961

17 Howie J.B & Helyer B.J Ann. N Y Acad. Sci. 1 4 167 1965

18. Larsen, R.A. & Boyum, A.. Acta med. scand. Suppl. 543 p. 21 1972.

19 Larsen, R.A. Acta med. scand. Suppl. 543 p 11 197..

20 Leonhardt, T.. Acta med. scand. Suppl. 416 1964.

21 Mell rs, R.C. & Huang, C.Y J exp. Med. 1 4 1031 1966.

2* Norms, L.C. & Holmes, M.C. J Immunol. 93 148 1964.

23 O Brien, W.M. Clin. exp Immunol. 2: 785 1967

24. O'Brien, W.M., Bennett, P.H., Burch, T.A. & Bunim, J.J Arthr and Rheum. 10 163 1967

25 Rodnan, G.P., Machlachlan, M.T & Creighton, A.S.. Clin. Res. 8 197 1960.

26. Smith, C.A.B Ann. hum. Genet. 20 257 1956

27 Smith, C.A.B.: Ann. hum. Genet. 23 311 1959

28. Snedecor G W & Cochran, W G Statistical Methods, pp. 253–257 Sixth Edition. The Iowa State University Press, Ames, Iowa, U.S.A. 1967

29 Solheim, B O & Larsen, R.A.. Acta med. scand. Suppl. 543 p. 43 1972.

30 Stevens, M.B., Abbey H. & Shulman, L.E. New Engl. J Med. 268 976 1963

31 Svec, K.H. & Kaplan, M.H. Arthr and Rheum. 6 11 1963

32 Svec, K.H. Arthr and Rheum. 9 545 1966.

33 Vasey H. & Fallet, G.H. Z. Rheumaforsch. 22 429 1963

34. Wldelock, D Gilbert, G., Siegal, M. & Lee, S. Amer J publ. Hlth 51 829 1961

Family studies in systemic lupus erythematosus (SLE) Acta med. scand. Suppl. 543 p. 43 1972.

IV Presence of antinuclear factors (ANFs) in the total populations of relatives and spouses, and the correlation to rheumatic disease

BJARTE G. SOLHEIM & ROLF A. LARSEN

The Gade Institute Department of Pathology University of Bergen, Haukeland Hospital, Bergen, (present address Secti f Immunology Institute for Experimental Medical Research, Ullevaal Hospital, Oslo 1 Norway) and Section for Immunology Institute fo Experimental Medi al Research, University of Osl Ullevaal Hospital Osl 1 Norway

ABSTRACT

The distribution of antinuclear factors in sera of 615 relatives, and their 313 spouses, f 72 SLE probands is presented. A statistically significant, but irregular aggregation f ANF occurred in relatives. When the different patterns of nuclear fluorescence were analysed, the homogeneous type, but not th speckled type f ANF separated the relatives and the spouses. The correlation between ANF and rheumatic disease was more manifest for relatives than for spouses. Non-rheumatic relatives and spouses did not differ with regard to any type of ANF

Immunofluorescent techniques for demonstration of antinuclear factors (ANF) in SLE and allied disorders were described by separate research groups in 1957 (9 10 11 18 24) ANF are also present in non-rheumatic diseases (3) and in healthy individuals (4 13) ANFs react with different nuclear antigens (2 12, 25) and belong to different classes of immunoglobulins (1)

ANFs occur with increased frequency in relatives of patients with SLE but the prevalence f ANF in the families varies greatly (for references, see 7 17) This is partly due to variations in the techniques used, but different selection of SLE probands may also contribute to the variation. There are still no definite generally accepted criteria f r the diagnosis f SLE (20)

The present work is part of an extensive investigation f clinical and serological aberrations in relatives of SLE probands from central eastern Norway This paper deals with ANF in the t tal populations f relatives and their spouses and th correlation t rheumatic disease. In the accompanying paper (16) the occurrence f ANF in various populations of relatives f selected SLE probands is described.

MATERIALS AND METHODS

SLE probands: The criteria f selection of the 72 probands with SLE and their clinical data have been published elsewhere (14) Th probands represented all individuals considered t be definit SLE cases in defined area from central eastern Norway during the years 1959—1963 Fifty-one probands with SLE were available f r further blood studies, but the disease was not necessarily active during bloood sampling

R latives and their spouses: The sampling of relatives and spouses used as controls, as well as th age and sex distribution of th clinically and serologically examined relatives and spouses, have also been reported elsewhere (15) First degree relatives (children, siblings and parents) second degree relatives (siblings of parents) and spouses were primarily included in the investigation. »Other relatives« mainly

grandparents and grandchildren, were also included when available. The completion rates of the various groups of relatives varied from 86.6 to 98.5 per cent. The completion rates of the various groups of spouses varied from 82.4 to 91.4 per cent.

Statistical methods: The data were punched on data cards and processed in a CD 3300 computer at the University of Oslo, Norway. The age distribution usually differed in the various populations. ANF positivity is regarded as being agedependent (3 4 23). χ^2 calculations were therefore perf rmed separately for each age group and the total group (obtained by summing up the individuals in the various age groups) but significant differences (p values below 0.05) between total groups were usually not taken into account. When the task was to combine evidence f r the three age groups (a number of 2 x 2 tables) Mantel Haenszel's method (M–H.) was employed (22). A continuity correction was used (8 22).

All p-values of significant level (< 0.05) are given in the tables. However when the p-values in specified groups seem to contribute to significant differences when groups are summarized, on-significant p-values are also indicated. In some instances, non-significant p-values are also given.

Tests for ANFs.

(1) *Sera.* Sera of probands, relatives and spouses were stored in small aliquots at 22 C and thawed once before use. The sera were coded and tested by a double blind technique.

(2) *Conjugates.* Rabbit antisera against pepsin-split human IgG (19) were used t ensure demonstration of ANF of all immunogl bulin classes. The antisera were made 1.65 M in $(NH_4)_2 SO_4$. The precipitates were washed and then dissolved in distilled water of half the original volume of serum, and dialysed extensively against saline. Conjugation with fluorescein-iso-thio-cyanate (FITC) was performed using 15 mg FITC per g protein in carbonate bicarbonat b ff f pH 9.0 at 4 C overnight. L jug ed FITC was moved by gel filtratio o Sephadex G The onjugate was dil t d appro m h 8 imes th original t serum lume. Th dil ion consistently g good h moge fl scenc with fer n ANF serum dil d 1 4000. Controls for unspecif staining wer p t regular n l did not ur sorption of the conjugates was unnecessary.

(3) *Buffers:* Phosphate buffer (0.15 M KH_2PO_4 and 0.15 M $Na_2HPO_4 \cdot 2H_2O$) of pH 7.0 was used. For washing procedures and diluti n of the sera phosphate buffered saline (1 part phosphate buffer and 9 parts 0.9 per cent NaCl) was used.

(4) *Source of nuclei, incubation and microscopy.* Unfixed cryostat sections (5–10 μ thick) of fresh snap-frozen rat liver were air-dried at room temperature f 10–30 minutes, incubated with serum (undiluted and diluted 1/16) for 30 minutes, washed 3 times for 5 minutes by gentle agitation, incubated with conjugate for 30 minutes, washed 3 times for 5 minutes, and finally mounted with buffered (pH 7.0) glycerol-gelatin. Incubation and washing were performed at room temperature and all tests were made in duplicate. Positive and negative controls were included in each series. The sections were examined the same day by dark field ultraviolet light using a Zeiss microscope (primary filter Scott BG 12 and secondary filter 41–65 or 44–65).

Three distinct patterns of nuclear fluorescence were observed (2) homogeneous speckled and nucleolar. Sera which showed nuclear fluorescence when diluted 1/16 were set up in twofold serial dilutions, and the last dilution which gave a weak but distinct fluorescence determined the titre. These tests were also performed in duplicate. The degree of nuclear fluorescence was graded from + to ++++; one plus fluorescence was regarded as weak, and +++ and ++++ as strong fluorescence. Sera with titres of 1/16 and higher were considered as positive. ANFs were classified according to the patterns of nuclear fluorescence. The sera giving more than one pattern were classified according to the different patterns. Thus, the number of positive tests were grouped as follows:

(1) All patterns combined, i.e., all individuals with at least one pattern present. Nucleolar pattern was extremely rare and was therefore included in this group. (2) Homogeneous pattern, i.e. all individuals with homogeneous pattern even if other patterns were present. (3) Speckled pattern, i.e., all individuals with speckled pattern even if other patterns were present.

Th tests were performed during th years 1964–1966.

RESULTS

A. The prevalence of ANFs in SLE probands

ANFs (all patterns combined) were demonstrated in 37 (72.5 per cent) of the 51 SLE probands tested. Homogeneous and speckled ANF were f und in 58.8 and 33.3 per cent respectively Nucleolar pattern was rare and only observed in 3 patients.

B Th p evalenc f ANF' in elatives and spouses.

(1) *All patterns combined* Sera of 20.9 per cent of th relatives nd 13 7 per cent of the spouses were positive the age and sex distribution of ANF in relatives and spouses is presented in T bles I and II.

Table I. *ANFs determined by immunofluorescence* Frequency f positive tests (all patterns combined) in groups of relatives of 72 SLE probands.

Group	Sex	Age distribution (yrs) <40	40 – 59	≥ 60	Total
Off-spring	female	6 (17.6**)	0		6 (16 6)
	male	5 (12.8)	2 (40 0)		7 (15 9)
Siblings	female	4 (11 7)	17 (27 4)	5 (20 8)	26 (22.8)
	male	3 (13.6)	12 (20 0)	2 (10 0)	17 (26 6)
Parents	female		0	8 (29 6)	8 (22 8)
	male		2 (28 5)	9 (36 0)	11 (34 3)
First degree	female	10 (14 7)	17 (23.6)	13 (25 4)	40 (20 9)
	male	8 (13 1)	16 (22 2)	11 (24 4)	35 (19.6)
	both	18 (13.9)	33 (22.9)	24 (25 0)	75 (20 3)
Sib of mother	female	0	4 (17 3)	9 (20 4)	13 (19 1)
	male	0	3 (13.0)	5 (17.2)	8 (14 5)
Sib. of father	female		3 (27 2)	11 (42 3)	14 (37.8)
	male	0	4 (26.6)	6 (23.0)	10 (23.8)
Second degree	female	0	7 (20.5)	20 (28.5)	27 (25 7)
	male	0	7 (18 4)	11 (20 0)	18 (18.5)
	both	0	14 (19 4)	31 (24.8)	45 (22.2)
First and second degree	female	10 (14 4)	24 (22.6)	33 (27.2)	67 (22 6)
	male	8 (12 3)	23 (20.9)	22 (22.0)	53 (19.2)
Other	both	5 (20.8)	1 (11 1)	3 (27.2)	9 (20 4)
Total	both	23 (14 5)	48 (21.3)	58 (25.0)	129 (20.9)

* No of positive in the group
** Percentage of positive in the group

Table VII *ANF's determined by immunofluorescence.* Statistical analysis of the difference between relatives (of 72 SLE probands) with and without rheumatoid symptoms. Methods: χ^2-calculations and Mantel-Haenszel's method (M-H.). Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

			Age distribution (yrs)			
Group	Sex	ANF pattern	<40 df 1	40 – 59 df 1	≥60 df 1	All age groups combined M-H. two-tailed
First degree	male	all comb		0.20		0.082
	both	homogen.		0.406	0.79	
	male	speckled		0.31		0.064
	both	speckled		0.406	0.63	0.117
Second degree	both	all comb			0.067	
	both	homogen.		0.97	0.026	0.0387
	both	speckled		0.79	0.42	0.31
First and second degree	male	all comb		0.34	0.566	0.039
	female	homogen.		0.828	0.165	
	male	homogen.		0.206		0.151
	female	speckled		0.56	0.29	
	male	speckled		0.465	0.767	0.064
Total	both	all comb		0.67	0.19	0.20
	both	homogen.		0.48	0.185	0.069
	both	speckled		0.31	0.36	0.089

cent, respectively) male individuals showing highest differences. A statistically significant difference between rheumatic and non-rheumatic relatives was present for aged second degree relatives and total second degree relatives when all age groups were used. The difference between the total populations of relatives was not statistically significant (cf Table VII)

(3) Speckled pattern. This type of ANF and rheumatic variables showed a slight association. Rheumatic male first degree relatives had this type of ANF in 1 per ent ompared with 9.8 per cent m heumatic first degree males. However h differ between th two populations f rela s was t statistically significant.

Spouse

Rheumatic an m spouses did not differ m an l ANF

Relatives and spouses with rheumatic diseases:

Sera of rheumatic relatives contained ANFs more frequently than sera of rheumatic spouses, the degree of significance of the differences between rheumatic relatives and spouses was generally higher than for the total populations of relatives and spouses, mainly due to first degree males and second degree females, when all patterns were combined, and to second degree females when homogeneous ANF was considered. The results of some of the tatistical calculations ar shown in Table VIII. The frequency of ANF giving the speckled pattern did not differ significantly between rheumatic relatives and spouses.

Relatives and spouses without rheumatic disease

Non-rheumatic relatives and spouses did not differ with regard to any type of ANF

Table VIII. *ANF determined by immunofluorescence* Statistical analysis of the differences between relatives (of 72 SLE probands) and their spouses, all presenting evidence f rheumatic disease. Methods: χ^2 calculations and Mantel Haenszel' method (M-H.) Degrees of freedom (df) are indicated The figures show the p-values in the different age and sex groups.

Group	Sex	ANF pattern	Age distrib tion (yrs) <40 df 1	40 – 59 df 1	>60 df 1	All age groups combined M-H. two-tailed
First degree	female	all comb				
	male	all comb		0 115		0.054
	both	all comb		0 0957		
	female	homogen.		0 49		
	male	homogen		0 17		
	both	homogen.		0.08	0 38	
Second degree	female	all comb				0 0377
	male	all comb				0 257
	both	all comb			0 048	0 01
	female	homogen.				0 017
	male	homogen				
	both	homogen.		0 25	0 06	0 0157
First and second degree	female	all comb		0 19	0 089	
	male	all comb		0.098	0.38	0.0227
	female	homogen		0 126	0 054	
	male	homogen		0 169		0 099
T tal	both	all comb		0 0257	0 0246	0 0058
	both	homogen		0 029	0 024	0 0018

D. The orrelation between LE factor and ANF in relatives and spouses.

The prevalence of LE fact in relatives and spouses has been reported elsewhere (14) Fifty two (69.3 per cent) out f 75 relatives with homogeneous ANF had LE factor; th percentage in spouses was 37.5 per cent. The correlati n was highest in female first degree relatives (95 7 per cent) LE fact and speck led ANF were n t related, the percentages in relatives and spouses being 31 7 and 22.9 respectively

E. ANF and siz f sibships and families.

Two hundred sibships with 571 individuals occurred in first and second degree relatives. The sibship size varied from one to ten when the SLE probands were n t considered. No apparent increase f ANF by size f sibships was noticed, e.g homogeneous ANF was pre sent in 28.5 per cent of the sibships: once in 23 per cent, twice in 4.5 per cent, three times in 0.5 per ent and four times in 0.5 per ent. In one sibship of eight four members presented homogeneous ANF

The family size, including first and second degree relatives, varied from one t eighteen when the SLE probands were not considered. N apparent increase f ANF by size of families was noticed, e.g., homogeneous ANF was f und in 58.3 per cent f the families: once

in 40 1 per cent, twice in 4.2 per cent, three times in 9 7 per cent, four times in 1 4 per cent, etc. In one family of sixteen, six members presented homogeneous ANF

Th distribution of ANFs in sibships and families was calculated and found consistent with the Poisson distribution.

F ANFs and the residence of relatives and spouses.

One fifth of the relatives and spouses lived outside the area in which the SLE probands were diagnosed (15) The prevalence of ANFs did not differ in relatives and in controls living outside and inside this area.

DISCUSSION

A negative ANF test usually makes the diagnosis of definite SLE unlikely (for references, see 5) In the present material, ANFs were demonstrated in 37 (72.5 per cent) of the 51 SLE probands tested. However those who had negative ANF tests exhibited no disease activity when the blood samples were taken. W re garded this to be the explanation as the presence of antinuclear antibodies, especially anti-DNA, and other antibodies in SLE is correlated to disease activity (21)

The prevalence of ANFs was determined in 615 relatives, and their 313 spouses, of 72 SLE probands from central eastern Norway Three distinct patterns of nuclear fluorescence (homogeneous, speckled and nucleolar) were observed, but the nucleolar pattern was rare.

W have demonstrated an aggregation of ANFs in the relatives of the SLE probands when compared with their spouses who served as controls, b t the distribution of the different types of ANF was ery irregular As rule ANF occurred m re frequently in all groups of relatives above forty year of age Age-dependent frequen [illegible] of ANF is even more pronounced in normal [illegible] (4) and then mostly in fem[illegible] (3 [illegible] 3

Female rela[illegible] es sh[illegible] w[illegible] h[illegible] highest frequencies of AN[illegible] [illegible] moderate and irr[illegible]ular Th[illegible] ype was most frequent, b[illegible]h in [illegible] pouses, with the exception of young females (highest frequency in spouses) there was no significant difference between relatives and spouses. By contrast, ANF giving the homogeneous pattern clearly separated the two populations, mainly due to females. Male relatives did not differ significantly from male spouses.

The relatives and the spouses differed in other ways. the correlation between ANF and rheumatic disease was more conspicuous for relatives than for spouses. Rheumatic and non-rheumatic relatives generally differed in ANF most distinctly for young male first degree relatives (speckled type) and second degree relatives (homogeneous type) By contrast, rheumatic and non-rheumatic spouses did not differ in any type of ANF This was also noticed for non-rheumatic relatives and spouses. LE factor and homogeneous ANF were better correlated in relatives than in spouses.

The cause of the irregular aggregation of ANFs in relatives of SLE probands is obscure There was no increase of ANFs with sibship or family size and ANFs occurred with similar frequencies in family members who had lived far from each other for many years. The irregular ggregation of ANFs in relatives may indicate that different populations of relatives with varying prevalence of ANFs actually oc cur Different clinical symptoms may predominate during active disease in individual patients with SLE. We have therefore selected SLE probands on clinical, serological, or combined clinical-serological basis the results of tests for ANF in the various populations of relatives of these selected SLE probands will be present d in the accompanying paper

REFERENCES

1 Barnett, E.V., Condemi, J.J., Leddy J.P & Vaughan J.H.. J clin. Invest. 43 1104 1964.
2. Beck, J.S Lancet I 1203 1961
3 Beck, J.S Scot. med. J 8 373 1963
4 Cammerata R.J., Rodnan, G.P & Fennell R.H. J Amer med. Ass. 199 455 1967
5 Christian C.L. Arthr and Rheum. 11:588 1968

6. Committee of the American Rheumatism Association 1958 Revision of Diagnostic Criteria for Rheumatoid Arthritis. Bull. rheum. Dis. 9 175 1958
7 Dubois, E.L. In Dubois, E.L. ed. Lupus Erythematosus pp. 129–276 McGraw-Hill Book Company New York 1966.
8 Dunn, O.J : Basic Statistics A Primer for the Biometrical Sciences, pp 1 1–125 John Wiley & Sons, Inc., New Y k 1964.
9 Friou G.J., Finch, S C. & Detre K.D J Immunol. 80 324, 1958
10. Holborow E.J., Weir D.M. & J hnson, G.D Brit. med. J 2 732 1957
11 Holman, H.R. & Kunkel, H.G Science 126 162 1957
12. Lachmann, P.J & Kunkel, H.G : Lancet 2 436 1961
13 Laffin, R.J Bardawil, W.A., Pachas, W.N & McCarthy J.S Amer J Path. 45 465 1964.
14. Larsen, R.A.: Acta med. scand. Suppl. 543 p. 11 1972.
15 Larsen, R.A. Acta med. scand. Suppl. 543 p 31 1972
16. Larsen, R.A. & Solheim, B.G Acta med. scand. Suppl. 543 p 55 1972.
17 Leonhardt T Acta med. scand. Suppl. 416 1964
18 Mellors, R.C., Ortega, L.G Noyes, W F & Holman, H.R. J exp Med. 106 191 1957
19 Osterland, C.K., Harboe M. & Kunkel, H.G Vox Sang. 8 133 1963
20 Report from the subcommittee on diagnostic criteria for systemic lupus erythematosus. In Bennett, P.H. & Wood, P.H.N eds. Population Studies of the Rheumatic Diseases, Proceedings of the third international symposium, New York, 1966 pp. 287–289 I ternational Congress Series No 148 Excerpta Medica F undation, Amsterdam 1968
21 Schur P.H. & Sandson, J New Engl. J Med. 278 533 1968
22. Snedecor G W & Cochran, W G Statistical Methods, pp 253–257 Sixth Edition. Th I wa State University Press, Ames, Iowa U.S.A. 1967
23 Svec, K.H. Arthr and Rheum. 9: 545 1966.
24 Vazquez, J.J & Dixon, R.J : Lab.Invest. 6 205 1957
25 Tan E.M. J Lab clin. Med. 70 800 1967

V Presence of antinuclear factors (ANTFs) in and spouses of selected SLE probands

ROLF A. LARSEN & BJARTE G SOLHEIM
Section for Immunology Institut for Experimental Medical Research
University of Oslo Ulleval Hospital Oslo and
the Gade Institut Department of P thology University of Bergen
Haukeland Hospital, Bergen Norway

ABSTRACT

Antinuclear factors (ANF) were studied in sera of 81 SLE probands, 616 relatives and their 313 spouses who served as controls.

The frequency of ANF in the relatives was correlated t certain variables present in the SLE probands: A ery marked accumulation of speckled ANF was found in relatives of SLE probands with marked arthritis, whose female relatives above forty years of age more frequently had present of past history of effusions in large joints than female relatives of SLE probands without arthritis. The frequency f ANF in relatives was also correlated with la the titer of ANF in the proband and with m the presence of numerous LE cells at the time of diagnosis f SLE in the proband.

Selection f subroups f SLE probands with defined clinical and/or serological characteristics appears t be valuable in family studies of SLE.

The prevalence of antinuclear factors (ANFs) is increased in relatives f patients with SLE (for references, see 5 16) Varying frequencies in the different family materials are probably due to variations in the immunofluor escent methods used and in th selection f probands. W have confirmed th aggregation of ANFs in relatives of SLE probands in N rwegian material (18)

The distribution of antinuclear fact rs in the relatives was irregular This may indicat that different populations f relatives with varying frequency f ANF actually occur Different clinical symptoms may predominat during active disease in individual patients with SLE (13) and definit criteria for the diagnosis of SLE have not yet been established (17) W have therefore selected SLE probands n a clinical, serological, or combined clinical-serological basis. The corresponding population of relatives showed marked differences i occur rence of ANFs. The results of these studies are presented in this paper

MATERIALS AND METHODS

SLE probands, relatives and spouses. The criteria for selection f the probands with SLE, the mode f collecting sera and clinical data, the age and sex distribution, and the compl tion rates have been reported elsewhere (13 14)

Statistical methods: Th methods used have been described previously (18)

Tests f ANFs: The immun fluorescent method for demonstration of antinuclear fac tors and the criteria for positive reactions have been described in detail in the preceding paper (18)

Principles f selectio of subgroups f SLE probands: The subgroups f the SLE probands were selected on the basis of th f llowing characteristics presence of lupus nephritis, hypergammaglobulinemia, acute or chronic lupus kin lesions, numerous LE cells at the time of diagnosis, severe arthritis, and wire-loops (13) Fifty-one SLE probands were also classified according to the titers of ANFs. Finally

combined clinical characteristics and titer of ANFs were also used as criteria.

Comparison was first made between the frequency of ANFs in the relatives of SLE probands with and without certain feature This was followed by comparison of th fre quency in the corresponding spouses who ser ved as controls. In the subsequent text, only those subgroups of SLE probands are described in which the corresponding relatives showed a significant increase of ANFs.

RESULTS

A. Variable for selection. Marked arthritis in the SLE proband.

SLE probands were classified according to ARA s criteria for rheumatoid arthritis (RA) (4) SLE probands who developed RA features during their disease manifested these properties very early and initially showed a high incidence of effusions in small (finger and toe) and large (wrist elbow shoulder knee and ankle) joints (13) Twenty-four (33.3 per cent) of the SLE probands showed predominating symptoms of arthritis.

ANFs in relatives:

The proportion of relatives of the two groups of SLE probands was 1:2.

(1) All patterns combined: ANFs were de monstrated in 27.2 per cent of first degree relatives of SLE probands with marked arthritis and in 16.9 per cent of first degree relatives of probands without arthritis. A similar difference was found between second degree relatives. The distribution is seen in Table I

Some of the results of the statistical calculations are presented in Table II.

The relatives of SLE probands with marked arthritis more frequently had ANFs. Statistically significant differences between the two types of relatives were found for aged females. The degree of significance was higher when all age groups or first and se ond degree relatives were combined and used m the calculations. Significant differences were still present when both sexes were combined

(2) Homogeneous p t rn Sera giving homogeneous nuclear pat ern wer equally distributed in the two popul t relatives.

(3) Speckled pattern Sera of *female* relatives of SLE probands with marked arthritis gave speckled nuclear fluorescence about four times as often as the sera of female relatives of SLE probands without arthritis. There was a female preponderance of speckled ANF in relatives of SLE probands with arthritis, but the sex distribution of positive tests was reversed in relatives of SLE probands without arthritis. There was no marked age variation of speckled ANF in the populations. The distribution is shown in Table III

The results of statistical calculations are shown in Table IV

Statistically significant differences between the two groups of relatives were seen in the highest ge groups and when all age groups were combined. The differences were mainly due to female first degree relatives. However the degree of significance was higher when first and second degree relatives were combined. When both sexes were combined, the significant differences were still present. The difference f speckled ANF in the total populations of relatives was highly significant.

ANFs in spouses.

We did n t record any differences in frequency of ANFs in the orresponding two populations of spouses.

Clinical variables in relatives:

The frequency of ARA s criteria for RA and *of joint effusions in female relatives is shown in* Table V

Male relatives did not differ A past or present history of effusions in large joints occurred significantly more frequently in female relatives of SLÉ probands with marked arthritis than in female relatives of SLE probands without arthritis (using χ^2-calculations, $p < 0\ 005$ df 1) The frequency of effusions in large joints in the corresponding two populations of spouses was not different. The distribution of sera giving homogeneous nuclear fluor escence in the two populations of female relatives with ffusions in joints was different About half of th sera of female arthritic relatives of SLE probands with arthritis, versus only 11 per cent of female arthritic relatives of SLE probands without arthritis showed homogeneous nuclear fluorescence Speckled ANF occurred irregularly and was n t related to joint effusions in female relatives.

Table I *ANFs determined by immunofluorescence* Frequency of positive tests (all patterns combined) in relatives of SLE probands with and without marked arthritis.

Arthritis in SLE probands	Group of relatives	Sex	Age distribution (yrs) <40	40 – 59	≥ 60	Total
Present	Siblings	female	1 (14.2**)	9 (31.0)	3 (25 0)	13 (27 0)
Absent		female	3 (11 1)	8 (24 2)	2 (16 6)	13 (18.0)
Present	First degree	female	3 (16 6)	9 (30 0)	9 (40.9)	21 (30 0)
		male	1 (7 6)	6 (26 0)	5 (33 3)	12 (23.5)
		both	4 (12.9)	15 (28 3)	14 (37 8)	33 (27.2)
Absent		female	7 (14.0)	8 (19.0)	4 (13 7)	19 (15 7)
		male	7 (14.5)	10 (20.4)	6 (20 0)	23 (18.1)
		both	14 (14.2)	18 (19 7)	10 (16.9)	42 (16.9)
Present	Second degree	female		1 (33.3)	10 (47.6)	11 (45.8)
		male	0	2 (28 5)	5 (23 8)	7 (24 1)
		both	0	3 (30.0)	15 (35 7)	18 (33 9)
Absent		female	0	6 (19.3)	10 (20 4)	16 (19 7)
		male	0	5 (16 1)	6 (17.6)	11 (16.1)
		both	0	11 (17 7)	16 (19 2)	27 (18 1)
Present	First and second degree	female	3 (16 6)	10 (30 3)	19 (44 1)	32 (34 0)
		male	1 (7 1)	8 (26.6)	10 (27 7)	19 (23 7)
Absent		female	7 (13 7)	14 (19 1)	14 (17.9)	35 (17.3)
		male	7 (13 7)	15 (18 7)	12 (18 7)	34 (17 4)
Present	Total	both	7 (15 5)	18 (26.0)	30 (34.8)	55 (27.5)
Absent		both	16 (14 1)	30 (19 2)	28 (19 1)	74 (17.8)

* No of positive in the group
** Percentage of positive in the group

B Variables for sele tion: High-titered (titer ≥ 1/256) versus negative tests for particular type f ANF in the SLE probands

Th famili f these two categories f SLE probands were compared to investigat whether the pattern of ANF in the SLE probands and the relatives were correlated.

(1) All patterns combined

Seventeen SLE probands had high titers of this type f ANF and fourteen SLE probands were negative.

Relatives One hundred and fifty five and 102 relatives belonged t the two populations, i.e relatives f high-titered and negative pro-

Table IV *ANF's giving a speckled pattern in immunofluorescence*. Statistical analysis of the differences between relatives of SLE probands with and without marked arthritis. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in the different age and sex groups.

Group	Sex	Age distribution (yrs) <40 df 1	40 59 df 1	≥60 df 1	All age groups combined M-H. two-tailed
Siblings	female		0 044		0 06
First degree	female		0 047	0 11	0 001
	male				
	both		0.04	0 03	0.002
Second degree	female			0 16	0 08
	male				
	both		0.2	0.2	0.046
First and second degree	female		0 013	0.015	<0.0001
	male			0.32	0.2
Total	both		0 024	0 02	0.0006

Table V *Rheumatoid symptoms* (a past or present history) in female relatives of SLE probands with and without marked arthritis.

Arthritis in SLE proband	Symptoms in relatives		Years of age <40	≥40	Total
present	No. of ARA criteria for RA	2	2 (6.8**)	7 (8 1)	9 (7 8)
absent			4 (6 9)	12 (7.5)	16 (7 4)
present		3 –4	*2 (6 8)*	*10 (11.6)*	*12 (10 4)*
absent			0	13 (8.2)	13 (6 0)
present		5 – 6	0	5 (5 8)	5 (4.3)
absent			0	4 (2.5)	4 (1 9)
present	Effusions in	small joints	0	8 (9.3)	8 (7 0)
absent			0	9 (5 7)	9 (4.2)
present		large joints	2 (6.8)	11 (12.8)	13 (11.3)
absent			0	6 (3 7)	6 (2.8)

No. of affected individuals in the group
** Percentage of affected individual in the group

Table VI. *ANFs determined by immunofluorescence* Frequency of positive tests in relatives of SLE probands with high titers of ANF and negative tests for ANFs.

ANFs in SLE probands	Group of relatives	Sex	Age distribution (yrs) <40	40 – 59	>60	Total
high titer	Second degree	female		2 (100)	11 (42.3)	13 (46.4)
		both	0	4 (80.0)	14 (33.3)	18 (38.2)
negative		female		0	1 (7.6)	1 (6 6)
		both		1 (14.2)	3 (13.6)	4 (13 7)
high titer	First and second degree	female	2 (12.5)	8 (33.3)	15 (42.8)	25 (33.3)
negative		female	2 (18.1)	4 (25.0)	3 (13.0)	9 (18.0)

The figures are recorded as in Table I.

Table VII. *ANFs determined by immunofluorescence:* Frequency of positive tests in relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis.

LE cells in SLE probands	Group of relatives	Sex	Age distribution (yrs) <40	40 – 59	>60	T tal
numerous	First degree	female	8 (19.5)	14 (25.9)	8 (24.2)	30 (23 4)
		male	6 (15.3)	15 (28.3)	9 (28 1)	30 (24 1)
		both	14 (17.5)	29 (27 1)	17 (26.1)	60 (23.8)
few		female	2 (8.0)	3 (16.6)	4 (23.5)	9 (15.0)
		male	2 (9.5)	1 (5.2)	1 (8.3)	4 (7.6)
		both	4 (8.6)	4 (10.8)	5 (17.2)	13 (11.6)
numerous	First and second degree	female	8 (19.5)	20 (25 6)	26 (29.5)	54 (26.0)
		male	6 (14.2)	18 (23 6)	16 (22.2)	40 (21.0)
few		female	2 (7 6)	4 (14.2)	6 (20 0)	12 (14.2)
		male	2 (9.0)	5 (14 7)	4 (15.3)	11 (13 4)
numerous	Total	both	17 (16 6)	39 (23.9)	45 (26 4)	101 (23.2)
few		both	6 (11.3)	9 (14.5)	10 (17.5)	25 (14.5)

The figures are recorded as in Table I

Table VIII. *ANFs giving a speckled pattern in immunofluorescence.* Frequency of positive tests in relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis.

LE cells in SLE probands	Group of relatives	Sex	Age distribution (yrs) <40	40 – 59	>60	T tal
		female	4 (9 7)	8 (14.8)	5 (15 1)	17 (13.2)
numerous		male	6 (15.3)	11 (20.7)	6 (18 7)	23 (18.5)
		both	10 (12.5)	19 (17 7)	11 (16.9)	40 (15 8)
	First degree	female	1 (4 0)	1 (5.5)	2 (11 7)	4 (6 6)
few		male	2 (9.5)	0	1 (8.3)	3 (5 7)
		both	3 (6 5)	1 (2.7)	3 (10.3)	7 (6.2)

The figures are recorded as in Table I

was not tested for LE-cells at the time of diagnosis, and had to be excluded.

Relatives·

Four hundred and thirty-five and 172 relatives corresponded to th two categories of SLE probands.

(1) All patterns combined: The proportion of positive tests for ANF was 2 1 in the two populations. The distribution is presented in Table VII.

Some of the statistical calculations are shown in Table VIII.

A significant difference was found for male first degree relatives when all age groups were used in the calculations, but the degree of significance was higher for all first degree relatives. The difference in female first and seco d degree relatives was not significant. However the difference between th total populations of relatives was statistically significant.

( ) Homogeneous pattern The distribution of this type of ANF was very irregular with no statistically significant differences between the populati ns (cf. Table VIII)

(3) Speckled pattern Th proportion of speckled ANF in the two populations of relatives was 1 The distribution in first degree relatives shown in Table IX.

Th statistical calculations are included in Table VIII Statistically significant differences were found betw en middle-aged first degree relatives, and between first degre relatives when all age groups were combined and used in the calculations.

Spouses

No statistically significant differences were observed.

DISCUSSION

We observed that the ccumulati n of ANFs in th relatives of probands with SLE was correlated to clinical and serological findings in the probands.

Speckled ANF appeared to be nearly equally distributed in relatives and spouses of the total proband material (18) but sera of relatives of SLE probands with and without marked arthritis gave different fluorescence Speckled antin clear factor occurred more frequently in relatives of SLE probands with arthritis, the aggregation was statistically significant both in first and second degree relatives, and was mainly due to females.

Speckled ANF is known to react with phosphate buffer extract of nuclei (12) and occurs in SLE and allied syndromes (?) The pattern of nuclear staining is more obvious in tumour imprints, and so-called true speckled staining i present in scleroderma and Raynaud phenomena (3) Rayna d phenomena occurred with increased frequency in the SLE probands with marked arthritis (13) and it is possible

Table IX. *ANFs determined by immunofluorescence* Statistical analysis of the difference between relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis. Methods: χ^2 calculations and Mantel-Haenszel method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	ANF pattern	Age distribution (yrs) <40 df 1	40 – 59 df 1	≥60 df 1	All age groups combined M-H. two-tailed
First degree	male	Speckled		0 074		0.056
	male	All comb		0 079		0 027
	both	Speckled		0 045		0 028
	both	All comb		0.07		0 017
Second degree	female	Homogen.				0 087
	female	All comb				
First and second degree	female	Homogen.				0 087
	female	All comb				0 066
	male	All comb				
Total	both	Homogen.				0 091
	both	All comb		0.17	0.23	0 035

that an accumulation of speckled ANF in their relatives occurred on this basis.

Female relatives of SLE probands with marked arthritis symptoms had significantly higher frequency of ffusi ns in large j ints. Initially the SLE probands who developed marked arthritic symptoms presented very high frequency f joint effusions (13) Speckled ANF was not elated to joint effusions in female populations. The frequency of speckled ANF was increased in rheumatic male first degree relatives compared with non-rheumatic male first degree relatives (18) but the frequencies f ARA criteria f RA and f joint effusions did t differ in male relatives of SLE probands with and without marked arthritis.

The prevalence f homogeneous ANF was similar in relatives f SLE probands with and without marked arthritis, but mbination f homogeneous uclear staining and present or past hist ry f joint effusions occurred more frequently in female relatives f SLE probands with marked arthritis than in female relatives of probands without arthritis.

An aggregation of rheumat id arthritis (RA) and of rheumatoid factors (RF) is also present in families of highly erosive RA, and RF is aggregated as continuous variable (15) Definite RA patients from hospitals and population surveys differ in frequency f RF and ANFs, which seem to b closely related in the latter type of population (9) However an accumulation of RA and RF does not occur in relatives o spouses f seropositive definite classical RA patients from hospitals (19) Two kinds of ANF are observed in the sera of RA patients: antibodies which give homogeneous nuclear fl orescence with nucleoprotein and liver nuclei, and antibodies which nly react with liver nuclei giving speckled nuclear staining (11) Antinuclear fact rs are present in synovial fluids and sera of RA patients (1 7) and granulocyte specific ANF are found to be associated with human RFs (6) The joint

deformities often differ in SLE and RA (10) In certain SLE patients a connection may exist between ability to produce speckled antinuclear factors and development of rheumatoid de formities in joints.

Antinuclear factors were related in SLE probands and their relatives, but the aggregation in relatives of SLE probands with high-titered ANFs was not particularly pronounced. However blood sample were taken for com parison of ANFs in probands with SLE whether or not the patients were in active stage of disease

Antinuclear factors (of speckled pattern and all patterns combined) occurred with higher frequency in first degree relatives of SLE probands with numerous LE cells at the time of SLE diagnosis than in relatives of probands with few LE cells at diagnosis. The frequency of ANFs in th corresponding populations of spouses did not, however differ significantly The aggregation was not due to the size of sibships or families investigated (18)

These findings supplement previous observa tions indicating that certain lupus features are related to the presence of particular antibodies e.g., complement fixing antinuclear antibodies in lupus nephritis (0) An association between clinical features of SLE and antinuclear anti bodies probably has to be sought at the time of SLE diagnosis. ANFs are obligatory in active SLE and the high prevalence of ANFs in relatives of SLE probands with marked arthritis or numerous LE cells at diagnosis may reflect a special power to produce a group or groups of antinuclear antibodies very extensively and these antibodies may somehow contribute to the phenomena used for selection of the probands.

The irregular appearance of ANFs in rela tives of probands with SLE may be interpreted as evidence of polygenic inheritance (8) which is also assumed for allied diso ders (16) The genetic mechanisms involved are probably very complex and remain to be elucidated. In family studies of SLE, selection of subgroups of probands with defined clinical and/or serological characteristics ppears to f cilitate this analysis.

REFERENCES

1 Barnett E.V., Bienenstock, J & Bloch, K.J., J Amer med. Ass. 198 143 1966.
2 Beck, J.S. & Hughes, P., Lancet ?:442 1966.
3 Burnham T.K., Neblett T.R. & Fine G Amer J clin. Path 50 683 1968.
4 Committee of the American Rheumatism Association: 1958 Revision of Diagnostic Criteria for Rheumatoid Arthritis. Bull. rheum. Dis. 9 175 1958
5 Dubois, E.L. In Dubois, E.L., ed. Lupus Erythematosus, pp 129–276, McGraw-Hill Book Company New York 1966
6. Elling P., Graudal, H & Faber V Acta med. scand. 18 : 707 1967
7 Elling, P., Graudal, H. & Faber V : Acta rheum. Dis. 7 225 1968
8 Falconer D.S Ann. hum. Genet. 31:1 1967
9 Feltkamp T.E W Idiopathic autoimmune diseases. Thesis, Amsterdam 1966.
10 Friedman H.H., Swartz, S., Trubek, M. & Steinbrocker O Ann. intern. Med. 38: 732 1953
11 Friou, G.J., Ehn M & Hill R.: Arthr and Rheum. 9: 505 1966.
12. Holman H.R. Deicher H.R.G. & Kunkel H.G : Bull. N Y Acad. Med. 35: 409 1959
13 Larsen, R.A. Acta med. scand. Suppl. 543 p 11 1972.
14. Larsen, R.A.: Acta med. scand. Suppl. 543 p 31 1972.
15 Lawrence J.S : Clin exp Immunol. 2 769 1967
16. Leonhardt, T Acta med. scand. Suppl. 416 1964
17 Report from the subcommittee on diagnostic criteria for systemic lupus erythematosus. In Bennett, P.H. & Wood, P.H.N eds. Population Studies of the Rheumatic Diseases, Proceedings of the third international symposium New York, 1966 pp. 287–289 International Congress Series No 148 Excerpta Medica Foundation, Amsterdam 1968
18 Solheim, B G & Larsen R.A.: Acta med. scand. Suppl. 543 p 43 1972.
19 Steiner F.J Veenhof-Germann, A.M Th., von Westendorp Boerma F & Valkenburg H.A. VI Congresso Europeo de Reumatologia Lisboa, 8A 13 de Outubro de 1967 p 8
0 Tojo T & Friou G.J : Science 161: 904 1968

Acta Medica Scandinavica

Supplementum 545

Early Diagnosis of Acute Myocardial Infarction

with Special Reference to the Diagnosis of the Intermediate Coronary Syndrome

A clinical study

By Urbain Sawe

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly. The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.

In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw. kr. 170, incl. postage.

Other countries Sw. kr. 200 incl. postage.

Chief Editor

Professor Jan Waldenström, MD
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Riddargatan 12 B
S-114 35 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksell Informationsindustri AB S-751 81 Uppsala, Sweden

EA[illegible]GNOSIS OF ACUTE MY[illegible]DIAL INFARCTION WITH SPECIAL REFERENCE TO THE DIAGNOSIS OF THE INTERMEDIATE CORONARY SYNDROME

— A clinical study —

by

URBAIN SAWE

STOCKHOLM 1972

Diagnosis is a matter of probability as those of us who follow the fate of our patients to post mortem room know only too well

Sir George W. Pickering

Acknowledgements

I want to express my sincere gratitude to all who have helped me during these studies, and especially to acknowledge:

Mrs Ingrid Säwe, my wife.

Professor Gunnar Biörck, whose genuine interest in cardiological research has encouraged me throughout my work. His positive criticism of the manuscript has been of great stimulation for me. He, also, as the Head of the Department of Medicine, Karolinska Institutet at Serafimerlasarettet provided the many facilities necessary for the progress and fulfillment of this work.

Erik Orinius, M.D. whose sharp and constructive criticism has been of great importance to my scientific education.

Andreas Sjögren, M.D. whose generous help with the revision of the manuscript including language corrections was of great value for me.

Olof Edhag, M.D. Torbjörn Lundman, M.D. and Lars Mogensen, M.D. who provided valuable help with their advice and criticism.

Leif Erhardt, M.D. and Olof Nyquist, M.D. whose friendship and encouragement have been essential.

Kurt Bergström, M.D., who enabled the enzyme analyses and also offered positive criticism.

Mr. Sune Carlsson, who gave valuable help in the statistical parts of this work.

Mr. Robert McAllister, who gave linguistic help.

Miss Karin Nordell, who provided valuable help with records and patients and was helpful in numerous other ways.

Miss Gunilla Bartholdson and her nursing colleagues at the coronary care unit who have greatly contributed.

Mrs Gunilla Haag, who skilfully typed and edited the final manuscript.

Mrs Kim Hellman, who gave enthusiastic help with the bibliography. Mrs Eva Haldén, who helped me with illustrations.

Mrs Ingrid Braunstein, Miss Lisbeth Gyllenstierna, Miss Rhode Jansson, Miss Ann Marie Lothigius, Miss Britt Rosén and Mrs Birgitta Öhman did secretarial help during these studies.

This investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases.

Contents

Previous History and Acute Symptoms in 921 Patients Admitted to a CCU

Acute central chest pain is the most prominent symptom in acute myocardial infarction. Yet pain with this location may be of other origins, and the interpretation of this symptom is an important problem in clinical medicine. Therefore with the introduction of specialized coronary care units (CCU) the interest of the symptomatology of acute myocardial infarction (AMI) has increased.

The diagnostic rate based on early appraisal is low today. Only half of the patients admitted subsequently fulfil the criteria for AMI (Lown et al. 1967, Wallace et al. 1967, Lundman et al. 1969, Amtorp et al. 1970, Askanas et al. 1970, Chiche et al. 1970, Engstedt et al. 1970, Haghfeldt, 1970, Asplund et al. 1971, Christiansen et al. 1971, Armstrong et al. 1972, Engstedt et al. 1972).

The difficulties of early diagnosis consist of the following:

1. The border region from the still normal to the already pathological condition.

2. The non-characteristic onset of numerous pathological entities with equivocal symptoms (Franke 1970).

TABLE 1 The criteria for admission to the CCU of Serafimerlasarettet, Stockholm, Sweden

1. Central chest pain lasting for more than 15 min within the last 48 h.
2. Frank pulmonary oedema without previous known valvular lesion, uremia or intoxication.
3. Shock without suspicion of acute hypovolemia or intoxication.
4. Syncope with electrocardiographic evidence of acute myocardial infarction.
5. Repeated bouts of chest pain with duration shorter than 15 min, without enzymosis, within the last 48 hrs.

The last two criteria were added on September 18, 1968.

The CCU of Serafimerlasarettet, Stockholm, has used well defined criteria for admission (Table 1). The cardiovascular history for each patient has been recorded in specially constructed registration charts in numerical form (Lundman et al. 1968). These two circumstances have allowed a comparison of the history and the acute symptomatology for 1) patients, who were subsequently diagnosed to have an AMI and 2) patients, who were found not to fulfil the diagnostic criteria (OBS) (page 13).

The purpose of this part of the present study was to penetrate the following questions:

1. Are there any differences in the past history or the acute symptomatology between patients with subsequently verified AMI and the remaining patients admitted to CCU on the same criteria?
2. Can these differences be combined to build a diagnostic index?
3. How many true negatives can be excluded without missing any true positives with such an index?

MATERIAL AND METHODS

From January 1, 1968 to January 1, 1970 1099 patients were treated in the CCU of Serafimerlasarettet. The organization of the CCU, general care, treatment of complication and after-care has been presented previously (Bäck et al. 1969). Of these patients 450 (41 per cent) fulfilled the criteria for a diagnosis of AMI. In 471 (43 per cent) this diagnosis could be excluded and in 125

TABLE 2 Admissions to the CCU in relation to the number of patients

	AMI		OBS	
	admissions	patients	admissions	patients
Admissions in both groups	88	69	99	69
More than one admission	50	23	80	34
One admission	31	312	792	292
Total	450	404	471	395

(11 per cent) the diagnosis was suspected acute myocardial infarction because of only partial ful fill ng the criteria. The remaining 53 patients were admitted for other reasons observation of ar rhytmias or complications of catheterization and so on.

The present study has been limited to a compari son of the past history and symptomatology n the 450 patients with acute myocardial infarction (AMI patients) and in the 471 patients in whom this diagnosis could be excluded. These latter pa ents who were observed in the CCU will be alled OBS-patients in the following.

Some patients were admitted more than once and as this study is based on admission to the CCU some patients are included in both diagnostic groups as s seen in Table .. Althogether 69 pa tients were represented in both groups and 3 patients were admitted more than once in the AMI group and 31 patients more than once in the OBS-group The remaining 312 AMI-patients and 79 OBS-patients were admitted once only The 9 1 admi sions therefore are made up of 730 in-d vidual

Adm p c d

Serafimerlasarettet is teaching hospital with 191 beds for general medic n Acut admissions are received in the Casualty Department where a phy sician on duty makes prelim nary ppraisal. If one of the criteria for admission (page 11) is fulfilled the patient is transported to the CCU without delay The same instruction is given to the staff of the general wards in respect of already hospitalized patients.

A junior and a senior physican are attached to the CCU during daytime and one physican is on duty at the unit during the rest of the week. About 20 different physicans specially interested in car diology have thus been connected to the unit during the time of the study

The history of the patients

At admission to the CCU all relevant data in the history of the patients were directly registered on specially constructed charts in numerical form (Lundman et al. 1968) for subsequent computer evaluation. After discharge the charts were checked against information from the hospital notes by the physicians attached to the unit. There are state ments available on each patient about time of onset, delay occurrence and type of pain, dyspnoea, clouding of the mind, unconsciousness, palpitation and vegatative symptoms at onset Furthermore documentation includes previous angina pectoris and/or myocardial nfarction. In all patients occur rence of previous hypertension, heart failure and diabetes mellitus is registered.

When patients gave uncertain information the code has been the negative for that parameter

Electrocardiography

Routine ECGs includ ng leads I II III, aVR, aVL, aVF CR_4R, CR_1 CR_2, CR_4 CR_5 CR_7 were taken with an ink jet recorder on admission and every subsequent morning during the CCU stay

Serum enzyme d term nations

Blood samples for enzyme determination were taken on admission and on the following mornings for at least 3 days. The following enzymes were routinely determined serum aspartate aminotransferase (S-GOT) serum alanine transferase (S-GPT) lactic dehydrogenase (LDH) and its isoenzymes, LD_1 and LD_2 a alphahydroxybutyrate

dehydragenase, (HBD) The analyses were performed in the Department of Clinical Chemistry at Serafimerlasarettet.

Criteria and definitions

Diagnostic criteria

The criteria for the diagnosis of AMI in the patients fulfilling the admission criteria to the CCU have been fulfilment of a, b or c.

(a) Appearance of a pathological Q wave and/or appearance or disappearance of a localized ST elevation followed by T inversion in at least two or more of 1 ECG-leads.

(b) Two S-GOT values of 40 U or more and with a maximum about 24 hrs after onset of symptoms in combination with lower S-GPT values with a maximum after about 36 hrs and/or two HBD values exceeding 75 % of corresponding LDH values higher than 400 U with maximum about 60 hrs after the onset of symptoms, or a combination of one S-GOT—S-GOT value and one HBD-LDH value elevated as stated above.

(c) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms.

Patients fulfilling the criteria for admission but not even partially the diagnostic criteria above have been classified as observation cases (OBS-patients)

Patients who only partly fulfilled the diagnostic criteria (e.g. one S-GOT value of 40 U/l or more, a localized ST elevation without T inversion etc) have been excluded from this study

DEFINITIONS

Past history

Angina pectoris—Angina pectoris was defined accordance with the WHO criteria. substernal pain with or without radiation or leftsided precordial chest pain radiating into the left arm. For both alternatives onset on exertion and disappearance within 10 min. of the patient resting or taking nitroglycerine was required (Rose, 1962)

Previous myocardial infarction—The requirement for acceptance of patient report of a previous myocardial infarction diagnosed at a hospital.

Previous heart failure—A history of digitalis therapy or diuretics therapy given for symptoms of heart failure.

Hypertension—A history of high blood pressure treated with antihypertensive agents.

Diabetes mellitus—A history of diabetes mellitus treated with antidiabetic agents.

Statistics—Age has been calculated from 10 year class means.

Significance of differences between mean values were tested by Student's t-tests. The Chi-square test was used for testing the significance of differences of relative numbers. Yates correction was applied when small numbers were employed. Degrees of significance were tested at the 5 1 and 0 1 per cent levels.

RESULTS

Sex and age distribution

Sex

There were 284 men (63 per cent) and 166 women (37 per cent) in the AMI-group giving a male- female ratio 1 7 1 In the OBS-group there were 274 men (58 per cent) and 197 women (42 per cent) giving a sex ratio of 1 4 1 This difference is not significant.

Age

Fig. 1 shows graphically the age distribution in men and women in the study The mean age was 66 years for the patients in the AMI group—63 years for men and 71 years for women. In the OBS-group of patients the mean age was 63 years—60 years for men and 67 years for women. The higher age for patients with AMI was significant for the entire patients groups ($p<0.001$) as well as for men ($p<0.01$) and women separately ($p<0.001$) This is emphasised by the findings that 9 men (3 per cent) in the OBS-group were below 40 years of age but none in the AMI-group. Twelve women (6 per cent) in the OBS-group were below 50 years of age against none of the women in the AMI group as is shown in Fig. 1

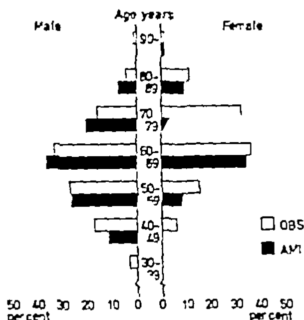


Fig. 1. Age distribution in relation to sex in the 450 AMI patients and the 471 OBS-patients

Comments

The sex ratio, i.e. males: females, is similar to previous Swedish studies from CCUs (Bevegård et al. 1970, Ahlmark et al. 1971, Boström and Ström 1971, Bronson and Malmcrona 1971, Henning and Holmberg 19 1). There is a tendency to female overrepresentation in the OBS-group compared with the AMI group but it does not reach statistical significance.

The mean age of the OBS-group was significantly lower for both men and women and this might therefore provide a factor of discriminant importance. In accordance age below 50 is more common in the OBS-group and age above 70 is more common in the AMI patients.

PAST HISTORY

History of previous angina pectoris and myocardial infarction

There was no difference between the AMI and OBS-groups concerning a history of angina pectoris of either short or long duration as can be seen in Table 3. In the AMI group the incidence was 59 per cent—54 per cent for men and 67 per cent for women—against 56 per cent in the OBE group—51 per cent for men and 62 cent for women.

In the AMI group 33 per cent of the patients had a history of previous myocardial infarction against 39 per cent in the OBS-group, the difference again not reaching statistical significance.

Neither of these two manifestations of previous IHD was noted in 33 per cent of the patients in the AMI group and also in 33 per cent in the OBS-group.

Comments

The incidence of previous angina pectoris and myocardial infarction was similar in the two groups. Therefore previous angina pectoris and myocardial infarction are not discriminant factors between the two groups. It is of interest that a

TABLE 3. History of previous angina pectoris (AP), myocardial infarction (MI) or none of these two IHD-manifestations in [illegible] AMI patients and [illegible] OBS-patients admitted to CCU

	AMI			OBS		
	Males [illegible]	Females [illegible]	Total n = 450	Males n = 2[illegible]	Females n = 197	Total n = 4[illegible]
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
From AP	[illegible]		59	51	6[illegible]	56
From AP less than 1 month	10	[illegible]	11	15	14	14
From MI	33	34	33	42	3[illegible]	39
None of AP or MI		[illegible]	33	[illegible]	30	33

[illegible]

TABLE 4 History of previous hypertension, heart failure and diabetes mellitus in 450 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
Hypertension	21	45	30***	6	31	17*
Heart failure	30	49	37*	19	57	35
Diabetes mellitus	7	16	10*	7	12	9*

N.S ** $p < 0.001$

third of the patients in both groups have no history of previous angina pectoris and/or myocardial infarction.

The incidence of previous angina pectoris varies in different AMI-materials ranging from 22 per cent to 73 per cent (Rosenbaum-Levine 1941) and the incidence of previous myocardial infarction from 16 per cent to 31 per cent (Biörck 1962 Bronson and Malmcrona 1971) The percentage of patients without any of these two IHD-manifestations is generally not given in the literature.

Engstedt et al. (1970) showed that 34 per cent in their series of OBS-patients had had angina pectoris and 17 per cent gave a history of myocardial infarction. Hofvendahl (1971) has pointed out that there are two different categories of patients in the OBS-group one with ischemic heart disease and one without and he also showed that none of the patients in the non IHD groups died within the first year after the discharge from the hospital, while the out look for the first year is rather the same for all CCU patients with previous IHD whether an AMI is diagnosed or not.

History of hypertension heart failure and diabetes mellitus

(as defined on page 13)

Table 4 shows the incidence of previous hypertension in the two groups of patients. Thirty per cent of the AMI-patients had a history of previous hypertension (21 per cent for men and 45 per cent for women) In the OBS-group the prevalence was 17 per cent (6 per cent for men and 31 per cent for women) The difference between the two groups is highly significant ($p < 0.001$) A history of previous heart failure was given by 37 per cent of the AMI patients against 35 per cent of the OBS-group. In this respect there was a difference between the sexes. Thirty per cent of the male AMI-patients reported previous heart failure against 19 per cent in the male OBS-patients which is significant. In the female patients there was no difference.

A history of diabetes mellitus was reported in 10 per cent of the AMI patients—7 per cent for men and 16 per cent for women—and in 9 per cent of the OBS-patients—7 per cent for men and 12 per cent for women.

Comments

The incidence of previous hypertension varies in different studies and definitions differ (Chambers 1946—Henning and Holmberg 1971) With the present definition—a history of high blood pressure treated with antihypertensive agents—this factor was found to be a discriminating parameter There is also a difference in this respect between the sexes within each group as well as when comparing men and women separately

Heart failure in the past history was a not differentiating factor between the two groups, nor was diabetes mellitus which was reported by the same percentage in the two groups.

To sum up history of previous treatment for hypertension is a discriminating factor between

TABLE 5 Time of onset of symptoms in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males	Females	Total	Males	Females	Total
	n = 84	= 166	n = 450	n = 74	= 197	= 471
	per cent	per cent	per cent	per cent	per cent	per cent
01—03	10	10	10	8	8	8
04—06	10	5	8	8	11	9
07—09	14	13	14	16	11	14
10—1	10	11	10	11	13	12
13—15	13	8	12	1	10	11
16—18	13	14	13	13	12	13
19— 1	16	18	16	14	15	14
2—00	11	15	1	12	12	1
Uncertain	1	6	5	6	7	7

the two groups of patients while heart failure and diabetes mellitus in the past are represented roughly equally in the AMI and OBS-group.

Time of onset of symptoms and delay between onset and admission

In Table 5 the time during the day of onset of symptoms is shown. Onset of symptoms was more uncommon during night time 1.00—6.00 am but this finding was similar in both groups of patients.

The delay between onset and admission was about the same in the AMI and OBS-patients as is shown in Table 6. A delay of less than 6 hours was however a little more common in the AMI patients in comparison to the OBS-patients ($p < 0.0$ [illegible]).

Comments

Neither the time of onset of symptoms nor the time interval between the onset of symptoms and admission to CCU does separate the two diagnostic groups (Table 5 and 6). This delay mainly depended on the time taken for decision of seeking medical help by the patient.

In the present study those patients with a delay for more than 48 hours were not admitted. About 75 per cent of all patients regardless of final diagnosis were in fact hospitalized within 3 hours.

SYMPTOMS AT ONSET

As the criteria for admission to the CCU are stringent the incidence of atypical symptoms in

TABLE 6 Time between onset of symptoms and admission to CCU in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males	Females	Total	Males	Females	Total
	[illegible]	166	45[illegible]	[illegible]	n = 197	n = 4[illegible]1
	per cent	per cent	per cent	per cent	per cent	per cent
3 hours	4[illegible]	[illegible]	41	39	39	39
6 hours	[illegible]	9	64	55	60	57
hours					77	

p [illegible] 05

TABLE 7 Chest pain before admission to CCU in 450 patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
Painless	6	7	6	8	8	8
Chest pain	27	16	23	33	31	32**
Chest pain with radiation	67	77	71	59	61	60*

*p.<0 01 ** p <0 001

acute myocardial infarction cannot be estimated. In Table 7 the number of patients admitted to CCU without pain but fulfilling the admission criteria is shown. Six per cent of the AMI-patients were admitted because of fulfilling criteria 2—4 (page 11) and 8 per cent of the OBS-patients were admitted in the same way

Chest pain

Table 7 shows the prevalence of patients with chest pain with or without radiation of pain in the two diagnostic groups. Pain without radiation occurred in 23 per cent of the AMI patients and in 32 per cent of the OBS-patients. Radiation of pain was reported by 71 per cent of the AMI patients and by 60 per cent of the OBS-patients. This latter difference is highly significant (p<0 001)

Comments

The radiation of pain to the arms is highly significantly more common in the patients with AMI compared to those admitted to CCU but found not to have AMI (OBS-patients) which has been shown in a prospective investigation (Sîwe, 1971) In the present study no further analysis of the distribution and radiation of pain could be done since in coding this parameter only occurrence of radiation has been noted. All patients, who have mentioned radiation from a central chest location to one or both arms, shoulders, neck and jaw back and to the epigastrium have been coded as having chest pain with radiation. A retrospective study of the details was therefore found impossible since the ordinary hospital records in most cases only give information of whether radiation of pain occurred or not. Still occurrence of radiation of pain was a discriminating factor between the AMI and OBS-patients.

Dyspnoea

Difficulty in breating following the onset of symptoms was registered by 44 per cent of the AMI patients and 51 per cent of the OBS-patients

TABLE 8 Dyspnoea before admission to CCU in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
No dyspnoea	58	51	56	55	41	49
Dyspnoea	31	35	32	36	44	39
Rattling respiration	11	14	12	9	15	12

TABLE 9 Disturbances in consciousness before admission to CCU in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males = 84 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
No disturbances of consciousness	83	76	80	79	71	77
Fainting	9	15	11	11	18	14
Unconsciousness	8	9	9	11	8	9

(Table 8) the difference not being significant. Twelve per cent in both diagnostic groups have noted rattling respiration.

Comments

About half of the patients mentioned that they were out of breath or felt shortness of breath previous to admission. At the registration of the description of this symptom complex, there was a separation of patients with dyspnoea and those with rattling respiration. These symptoms were similarly represented in the two groups of patients and therefore had no discriminating power.

Disturbances in consciousness

Eleven per cent of the patients in the AMI group had felt some clouding of their mind following the onset of symptoms as had 14 per cent of the OBS-patients (Table 9). This difference is not statistically significant. Unconsciousness was reported by 9 per cent of the patients in both groups.

Comments

In this study about 20 per cent of the patients regardless of the final diagnosis reported disturbances in consciousness in form of fainting or unconsciousness, and a history of these symptoms does therefore not separate the two groups of patients. In the literature the incidence of these symptoms at onset of AMI varies from 3 per cent (Chambers 1946) to 28 per cent (Bean 1938).

Arrhythmic sensation

Palpitation was reported by 29 per cent of the AMI patients—21 per cent in men and 41 per cent in women—as can be seen in Table 10. In the OBS-group palpitation was registered in 40

TABLE 10 Arrhythmic sensation before admission registered and reported by 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males [illegible] per cent	Females n = 166 per cent	Total = 450 per cent	Males 2 4 per cent	Females n = 197 per cent	Total = 4 1 per cent
Rapid irregular	[illegible]	14	8	9	11	11
Rapid regular	[illegible]	1	11	10	5	16
Extra beats			[illegible]	10	13	11
Slow regular or irregular		[illegible]	4	3	—	1
Arrhythmic sensation (combined)		41	29	[illegible]	52	[illegible]

p < 0.001

per cent—32 per cent in men and 52 per cent women. The difference between the two diagnostic groups was highly significant ($p<0.001$) as was the difference in this respect between the sexes regardless of diagnosis. The sensation of palpitation has been divided into rapid regular and irregular action of the heart, of extra beats and of regular and irregular slow action of the heart. The most common complaint of arrhythmic sensation was irregular rapid action of heart.

Comments

There is a discrepancy in the present study between the subjective registration of palpitation in the patients and objective findings in the acute phase of myocardial infarction. In this study arrhythmic sensations were reported significantly more often in the OBS-patients and this parameter has therefore a discriminating power. This was a surprise bearing in mind the high incidence of different arrhythmias during the initial stage of AMI. Arrhythmic sensations were more common in women than in men regardless of the final diagnosis.

Autonomic symptoms

The findings are presented in Table 11. Nausea, or feeling sick, as an isolated autonomic manifestation was reported by 11 per cent of the AMI patients and by 19 per cent of the OBS-patients. This difference was high statistical significance ($p<0.001$)

Vomiting, sweating and combination of vegetative symptoms were much more common in the AMI-patients when compared to the OBS-patients. These symptoms were present in 61 per cent of the AMI-patients and in 40 per cent of the OBS-patients ($p<0.001$)

Comments

Nausea, or feeling a desire to vomit as an isolated symptom of autonomic disturbance was more common in the OBS-group than in the AMI group while vomiting, sweating and combination of these symptoms were more common in the AMI group.

Nausea is a vague symptom meaning that patient mentions that he is unwell and feels sick while the other vegetative symptoms are more distinct and based on central mechanisms (Wang and Borison, 1950. Borison and Wang, 1953)

To sum up nausea isolated is a discriminating factor speaking more for the OBS-diagnosis while vomiting, sweating and combination of autonomic symptoms strongly speaks for the AMI-diagnosis.

DISCUSSION

With the introduction of the CCUs the problem of an early diagnosis of AMI has become of great importance. The accuracy of an early diagnosis of AMI is, however, low. Many patients are admitted to the CCUs with a symptom complex compatible with an AMI yet without subsequent verification of the diagnosis.

Lown, 1967 has discussed the problem with the policy of admission to the CCU. He considered that the challenge of maximal prevention requires

TABLE 11 Autonomic symptoms before admission in 450 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
No autonomic symptoms	29	26	28	44	35	41
Nausea feeling sick—only	10	13	11 **	18	21	19*
Vomiting, Sweating and combination of these symptoms	61	61	61	38	44	40*

$p<0.001$

TABLE 9 Disturbances in consciousness before admission to CCU in 430 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males n = 284 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
No disturbances of consciousness	83	76	80	79	74	77
Fainting	9	15	11	11	18	14
Unconsciousness	8	9	9	11	8	9

(Table 8) the difference not being significant. Twelve per cent in both diagnostic groups have noted rattling respiration.

Comments

About half of the patients mentioned that they were out of breath or felt shortness of breath previous to admission. At the registration of the description of this symptom complex, there was a separation of patients with dyspnoea and those with rattling respiration. These symptoms were similarly represented in the two groups of patients and therefore had no discriminating power

Disturbances in consciousness

Eleven per cent of the patients in the AMI group had felt some clouding of their mind following the onset of symptoms as had 14 per cent of the OBS-patients (Table 9) This difference is not statistically significant. Unconsciousness was reported by 9 per cent of the patients in both groups.

Comments

In this study about 20 per cent of the patients regardless of the final diagnosis reported disturbances in consciousness in form of fainting or unconsciousness, and a history of these symptoms does therefore not separate the two groups of patients. In the literature the incidence of these symptoms at onset of AMI varies from 3 per cent (Chambers 1946) to 28 per cent (Bean 1938)

Arrhythmic sensation

Palpitation was reported by 29 per cent of the AMI patients—21 per cent in men and 41 per cent in women—as can be seen in Table 10. In the OBS-group palpitation was registered in 40

TABLE 10 Arrhythmic sensations before admission registered and reported by 450 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males n = 284 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
Rapid regular	4	14	8	9	14	11
Rapid irregular	10	12	11	10	23	16
Extra beats	6	7	6	10	13	11
Slow regular or irregular	1	6	4	5	—	1
Arrhythmic sensation of any kind	1	41	29*	32	52	40* *

* $p < 0.001$

Computer Evaluation of Statistical Properties in the Early Differential Diagnosis of Patients Admitted to a CCU

The aim of part I was to study if there were any differences in the history of previous diseases and in the acute symptomatology between patients admitted to a CCU subsequently verified to have an AMI and those patients who did not fulfil the criteria of an AMI (the OBS-patients)

The aim of this part of the present study was to analyze the possibilities of working out a diagnostic index on basis of the results in part I.

The discriminative power of an index common to all patients has been evaluated through discriminative function analysis and its characteristics —sensitivity and specificity—are discussed.

MATERIAL AND METHODS

Parameters significantly discriminating between the 450 AMI patients and the 471 OBS-patients studied in part I are shown in Table 12 In seven parameters the chi-square value were more than 6.635 i.e $p<0.01$ Discriminant analysis was performed according to BMD 04 M (Dixon, 1965) The program used computes a linear function of number of variables measured on each individual. This function can serve as an index for discrimination between two groups.

The statistical theory and method has been described by Kendall and Stuart (1966) Discriminant analysis with this method has previously been applied to medical data in association with AMI (Hughes et al. 1963 Lemlich 1965 Pipberger et al. 1968, Shubin et al. 1968 Cohn et al. 1972, Cotton et al. 1972 and Nyquist 1972)

The discrimination ability is expressed by a F value. The higher this F value is the better is the discrimination between the two groups.

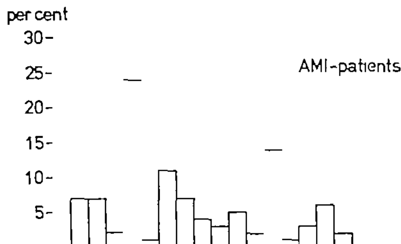
Sensitivity· i.e. the ability of a test to recognize the true positive and specificity i.e. the ability to find true negatives were calculated according to the following formulae (Cochrane and Holland 1971)

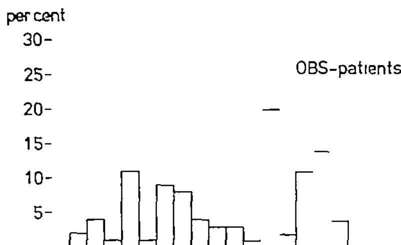
$$\text{Sensitivity} = \frac{a}{a+c} \times 100$$

$$\text{Specificity} = \frac{d}{b+d} \times 100$$

TABLE 12 Parameters significantly discriminating between 450 AMI-patients and 471 OBS patients

	AMI = 450 per cent	OBS = 471 per cent	P	X^2
Age <50 y	7	14	<0 001	11.33
>70 y	38	30	<0 01	7.80
Delay <6 h	61	57	<0 05	3 99
Hypertension	30	17	<0 001	22 00
Pain with radiation	71	60	<0 001	12 38
Arrhythmic sensations	29	10	<0 001	13 83
Nausea	11	19	<0 001	12.69
Vomiting, sweating and combination	61	40	<0 001	40 46





Radiation	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	−
Palpitation	−	+	−	−	+	+	−	−	+	+	−	−	+	+	−	+
Autonomic symptoms	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−
Hypertension	+	+	+	−	+	−	−	+	−	+	+	−	+	−	−	−

Fig 2 The discriminant scores of AMI- and OBS-patients, derived from the discriminant function analysis. The bars indicate the percentage of AMI and OBS-patients in each constellation of terms.

a. Individuals diagnosed as AMI and detected by the index (true positives)

b. Individuals diagnosed as OBS but positive to the index (false positives)

c. individuals diagnosed as AMI but not detected by the index (false negatives)

d. individuals diagnosed as OBS and negative to the index (true negatives)

Discriminant analysis was performed on 300 consecutive patients in each diagnostic groups (BMD 04 M) thereafter the procedure was repeated for all men and women separately and with exclusion of all patients without chest pain before admission.

RESULTS

Diagnostic index applicable to all patients admitted to CCU

This index was applied to all patients admitted to CCU

AMI

Radiation ✓		Palpitation ✓		
14	3	2	6	
24	11	3	7	↙Auton.sympt.
7	7	1	2	
4	5	1	2	↙Hypertension

OBS

Radiation ✓		Palpitation ✓		
20	12	4	14	
11	9	3	8	↙Auton.sympt.
2	4	1	1	
4	3	2	1	↙Hypertension

Fig 3 Two modified Venndiagrams showing the intersection between the four parameters used in the discriminant function analysis.

The highest F value (14.0) was obtained for the combination of the following parameters Radiation of pain arrhythmic sensation, autonomic (vegetative) symptoms (not nausea isolated) and a history of hypertension.

Combinations with the following three parameters with chi-square levels above 6.635 ($p < 0.01$) Isolated nausea, age below 50 years and above 70 years were excluded because of lower F values.

AMI OBS-ratio

4 1 -

3 1 -

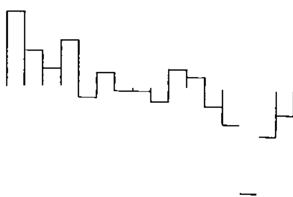
2·1 -

1 1 -

1 2 -

1 3 -

1 4 -



Radiation	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	−
Palpitation	−	+	−	−	+	+	−	−	+	+	−	−	+	+	−	+
Autonomic symptoms	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−
Hypertension	+	+	+	−	+	−	−	+	−	+	+	−	+	−	−	−

Fig 4 The ratio between AMI- and OBS-patients in each combination of the four parameters used in the discriminant function analysis.

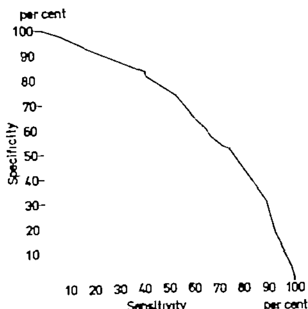


Fig 5 Specificity as function of sensitivity for the combination of the four items used in the test.

An attempt to find a more efficient separation of the two diagnostic groups through exclusion of all patients without chest pain before admission was not successful. Nor did discriminant function analysis for men and women separately improve index.

The calculated discriminant function coefficients

+0.00078 for radiation of pain
—0 00054 for palpitation
+0.00173 for combination of vegetative symptoms
+0 00092 for previous hypertension

The score (diagnostic index) for each patient was obtained by the following formula.

DI=0.00078 R —0 00054 P +0 00173 V +0 0009 H

where R = 0 if the patient is without radiation of pain and 1 if with radiation.

P = 0 if the patient has no palpitation and 1 if the patient has.

V = 0 if the patient has no vegetative symptoms and 1 if the patient has

H = 0 if the patient has no history of previous hypertension and 1 of the patient has.

The result of the analysis is presented in Fig. 2. The percentage of AMI and OBS-patients in each constellation of symptoms with decreasing score is presented in Fig 2 and in Fig. 3 two modified Venn-diagrams (Feinstein 1969) show the intersection between the four parameters used in the two diagnostic groups of patients. The ratio between AMI and OBS-patients in each combination of items is shown in Fig. 4.

Specificity at different levels of sensitivity was calculated for the combination of items and is graphically illustrated in Fig. 5

When the sensitivity of the test was 89 per cent the specificity was 30 per cent *meaning that* exclusion of about a third of the OBS-patients would mean a loss of 11 per cent of the AMI patients.

Comments

The result of this analysis shows that there is a difference of the combination of symptoms in the patients admitted to our CCU but there is a big overlap in the constellation of symptoms. A low index speaks against a diagnosis of AMI but its ability to exclude this diagnosis is very moderate. The risk of making wrong decision in the judgment of the individual patient may however be minimized if the ratio between AMI and OBS-patients in each combination of items is taken in account.

DISCUSSION

There was no complete separation of the two diagnostic groups of patients. The previous history and the acute symptoms differ between patients with AMI and patients without but there is a considerable overlap. The discrimination could perhaps be better if not only symptoms were taken in account but also clinical signs at admission, e.g respiratory rate, pulse pressure, systolic or diastolic blood pressure, heart rate and occurrence of rales. Another possibility of improvement of a diagnostic test would perhaps be if the results of some simple laboratory test at admission like the values of enzyme tests or an ECG were added.

Several authors (Lusted, 1965 and 1971 Sterling and Pollack, 1965 Bruce and Yarnall, 1966 Pipberger et al. 1968 Hall et al 1970 Björck, 1972) have recently discussed in some detail fundamental problems which arise when medical diagnosis is subjected to rigourous mathematical treatment. The result of this discriminant function analysis ought to be interpreted mainly as an attempt to identify the symptoms which are essential for the description of AMI and more important for the differentiation between AMI and OBS-patients. Which are the specific question which need to be asked when we want to arrive at a medical diagnosis? How much and what type of information is required

The findings in this part of the present study led to an attempt to improve the enzymatic diagnosis by more frequent enzyme determinations and a more detailed analysis of the previous history and the acute symptoms and signs in a prospective series of patients in order to bring all these informations together and try to improve the diagnosing of AMI in its early hospital stay

SUMMARY

The discriminant power of an index common to all patients has been evaluated through discriminative function analysis and its characteristics—sensitivity and specificity—are discussed.

The best discrimination was obtained for the combination of the following parameters. Radiation of pain, arrhythmic sensations, autonomic symptoms and a history of previous hypertension.

The analysis shows that there is a difference of the combination of symptoms in the patients admitted to the CCU but there is a big overlap. A low index speaks against a diagnosis of AMI but its ability to exclude this diagnosis is moderate. Exclusion of about a third of the OBS-patients would mean a loss of 11 per cent of the AMI patients.

This finding prompted prospective study with an improvement of the enzymatic diagnosis by more frequent enzyme determinations and a more detailed analysis of the previous history and the acute symptoms and signs in a consecutive series of patients admitted to CCU

Enzyme Diagnosis in Patients Admitted to a CCU with Special Reference to the Diagnosis of the Intermediate Coronary Syndrome

One of the reasons for a low diagnostic rate based on early appraisal could be an insufficient history-taking but another reason could be that some of the OBS-patients have a certain degree of myocardial damage not revealed by the ordinary routine of enzyme determinations as described in part I (page 12)

Since 1954, when LaDue and Wroblewski first measured the activity of serum glutamic oxalacetic transaminase in myocardial infarction, this and other enzymes have been widely used as diagnostic aids.

S-GOT CPK and heat stable LDH are now the chief ones used clinically and it is wise also to order a S-GPT determination to avoid a misinterpretation—at the presence of hepatic involvement ng elevated S-GOT values (Hughes 1969 Iberg and Winfield 1972)

The purpose of the present study was to attempt to improve the enzymatic diagnosis of acute myocardial infarction. Parts of the result of this investigation re being published together with Bergst öm (1972)

MATERIAL AND METHODS

The 191 patients described in part IV are the basis for this investigation The criteria for admission to the CCU ha e been presented in part I (page 11)

Blood samples were drawn on admission and on four further occasions (just before the meals) within the next 4 hour The blood s kept at 0 C and the serum sep ated w th n an hour Centr fugation and stor ce w perf rmed at −1 C The blood samples w e analyzed once daily using a Reactions Rate Analyzer (LKB 8600) connected to an evaluation unit (Optilab Bo Phi lip Instrumentation Stockholm) Reagents for S-GOT S-GPT and LDH were from KABI AB Stockholm. Heat stable lactate dehydrogenase (LDH) was measured as LDH after preincubation of serum and LDH reagents except for a-ketoglutarate at 65 C for 30 min. CPK was determined with a kit (Monotest from Boerhinger Mannheim, Western Germany)

Normal values for the enzymes used are pre sented in Table 13 (Bergström and Sävе, 1972)

DIAGNOSTIC CRITERIA BASED ON ENZYMES

The criteria for the diagnosis of AMI in this part of the present study have been the following: S-GOT values increasing to maximum above 40 U/l at more than one occasion 24—48 hours after onset of symptoms, simultaneously with or after an increase of CPK and following by a LDH curve with maximum later on and with a flat S-GPT curve At S-GOT values with high S-GPT the enzymatic diagnosis was confirmed by the CPK and LDH curves only

TABLE 13 Normal values for the enzyme used (Bergstrom and Sawe 1972)

Enzyme	Normal range	Borderline	Units
S-GOT	10— 35	35— 40	U/l (35 C)
S-GPT	10— 35	35— 40	U/l (35 C)
LD	100—350	350—400	U/l (35 C)
LD	50— 50	50—300	U/l (35 C)
CPK	10— 50	50— 80	U/l (35 C)

TABLE 14 Diagnosis of Acute Myocardial Infarction by using frequent enzyme analyses and comparison with the ordinary routine

Intervals of analyses during the first 24 hrs	Acute Myocardial Infarction		Intermediate Coronary Syndrome and Suspected Infarction		Observation Cases	
	n	per cent		per cent		per cent
Frequent (5/24 hrs)	88	46	19	10	84	44
Ordinary (2/24 hrs)	86	45	6	5	99	52

The criteria for the diagnosis of intermediate coronary syndrom (ICS) have been the following: the same type of S-GOT and LDH curves as seen in AMI but not exceeding the upper normal value (S-GOT = 40 U/l). The difference between the highest and lowest S-GOT value should be more than 10 U/l without changes in GPT and with a CPK curve typical for heart muscle damage. None of these patients had subsequently electrocardiographic evidence of typical AMI.

The criteria for the OBS-diagnosis have been the following: No fulfillment of the criteria for the AMI or ICS-diagnosis, i.e. normal constant enzyme values or other types of enzyme pattern.

RESULTS

AMI was diagnosed in 88 patients (46 per cent) and 19 patients (10 per cent) showed enzyme curves compatible with those found in AMI but with its maximum within the normal range giving them the diagnosis of ICS. In 84 patients (44 per cent) the enzymes were at a constant level or with an atypical pattern resulting in an OBS-diagnosis.

The differences in diagnosis obtained with the use of frequent serum analyses during the first 24 hours compared to the ordinary routine described in part I is shown in Table 14. Two patients who would have had a diagnosis of suspected infarction with ordinary routine were diagnosed as having AMI and 15 patients who would have been diagnosed as OBS-patients with the ordinary routine were diagnosed as ICS.

Different types of enzyme pattern in AMI

Most of the patients in the AMI group had typical enzyme curves but in some of the patients the pathophysiological course was modified. The S-GOT-maxima in relation to S-GPT maxima in the present study are shown in Table 15 a. In Table 15 b the CPK-maxima are shown.

TABLE 15 a. S-GOT and S-GPT-maximum in the three diagnostic groups

	AMI n = 88		ICS n = 19		OBS n = 84	
	S-GOT	S-GPT	S-GOT	S-GPT	S-GOT	S-GPT
≤20		11	—	14	28	51
21—30	—	21	7	4	33	13
31—40	—	25	9	1	10	7
41—50	3	11	3	—	2	4
51—100	30	14	—	—	7	7
101—150	17	2	—	—	1	—
151—200	18	1	—	—	1	
201—300	11	3	—	—	—	—
>300		—	—	—	—	—

TABLE 15 b CPK-maximum in the three diagnostic groups

	AMI = 88	ICS = 19	OBS = 84
<50	1	3	46
50— 80	1	6	17
81— 150	17	9	11
151— 300	29	—	4
301— 600	22	—	—
601—1000	8	—	—
>1000	1	—	—
Not obtained	9	1	6

A few patients did not develop an enzyme curve at once indicating, in retrospect, a pre infarction period without enzyme leakage followed by a typical AMI-curve later on.

In some patients often with repeated retrosternal pains, status anginosus, before admission the enzyme curves have more than one maximum, indicating a prolonged course with gradual extension of myocardial necrosis.

The transaminase values were sometimes difficult to interpret. High S-GOT values combined with high S-GPT were seen in some patients possible supporting a hepatic involvement. In these patients the diagnosis was confirmed by a typical CPK-curve and LDH-curve or by electrocardiographic evidence of an AMI.

Five AMI patients died within the first 24 hours in the CCU and in these patients no S-GOT maximum was reached, but the start of the enzyme curves in these patients were typical and the diagnosis was verified at autopsy either macro- or microscopically.

Diagnosis of the Intermediate Coronary Syndrome

Nine men and 10 women fulfilling our admission criteria to the CCU showed a typical curve pattern of S-GOT values not exceeding the normal range and with its maximum during the first 24 hours in CCU. In 7 of these patients the difference between the highest and the lowest value of S-GOT during the first 24 hours was 10—14 U/l and in the other 12 patients the difference was more than 15 U/l. The S-GOT and S-GPT-maxima for the ICS-patients are shown in Table 15 a and the CPK-maxima in Table 15 b. Two of the patients in this group showed ECG-changes as has been described by Prinzmetal et al. (1959) under the diagnosis of Variant Angina Pectoris. None of these 19 patients died during the stay in hospital.

Diagnosis of Observation case

Most of the patients in the group not fulfilling the diagnostic criteria for AMI or ICS showed constantly normal enzyme values, with a difference between the highest and lowest S-GOT value less than 10 U/l during the first 24 hours in CCU and in contrast to the ICS-patients these differences were not systematical. More than a fourth of the OBS-patients showed enzyme curves of different types mainly indicating a hepatic involvement with increased S-GOT and S-GPT values without a gradual increase to a defined maximum within the two first days at hospital. The enzyme maxima are shown in Tables 15 a and b.

DISCUSSION

Angina pectoris in the classical form described by Heberden and AMI in the equally classic picture of Herrick (1912) are the most well recognized clinical expressions of ischemic heart disease. Between these two, there is a condition that is more severe than angina and less severe than frank infarction, its significance has been uncertain and a source of controversy. This form of ischemic heart disease has been given a variety of names

1. Preliminary pain (Feil, 1937)
2. Impending infarction (Sampson and Eliaser 1937)
3. Coronary failure (Freedberg et al., 1948)
4. Intermediate coronary syndrome (Graybiel, 1951 Vakil, 1951)
5. Prodromal symptoms in myocardial infarction (Mounsey 1951)
6. Acute coronary insufficiency (Master et al 1956)

7 Premonitory pain (Maurice et al., 1956)
8. Preinfarction (Resnik, 1962 a, b)
9 Mikroinfarkt (Schneider 1968)
10. L infarctus rudimentaire (Delbecque 1969)
11 Acute coronary heart disease without recognizable infarction (Levine, 1971)

Intermediate coronary syndrome (ICS) is the term used throughout this study

Vakil and Graybiel independently recommended this term in 1951 Vakil (1961) stated that ICS appears to be a fairly common and recognizabl clinical entity usually selflimited, but with a proclivity to develop AMI in the near future

Gorlin (1972) stated that when the so-called intermediate syndroms between angina and infarction are suspected simply on the basis of severe chest pain and non-specific ST T-changes, the likelihood of the diagnostic accuracy is reduced and remains in doubt without further investigation.

Jennings et al. (1957) showed that the S-GOT content of the infarction area of the myocardium after a ligation of the circumflex artery in dogs decreases soon after the 20—30 min of ischemia necessary to induce irreversible injury in most fibers of the posterior papillary muscle and indicates that there must be defects of sufficient size in the semipermeable membrane of the f bers to allow an enzyme molecule (S-GOT) with an estimated molecular weight of about 60 000 to leak out.

Ruegsegger et al. (1958) stated from their study of experimental myocardial infarction in dogs that reversible myocardial damage without necrosis, such as that seen in coronary insufficiency and pericarditis, does not ppreciably influence the serum activity of S-GOT A cording to these authors the serum concentrations of GPT often remain within normal limits following smaller myoca dial infarcts in man.

In 1962 Resnik pointed out the possibility of very small myocardial necrosis i what he called preinfarction and stated that there s ev dence that in normal individuals, the maximal variat on between the lowest and highest figures of S-GOT obtained over a period of days does not exceed 10 units. Hence figures of 10 and 36 in the same patient probably have as much significance as a maximal level of 45 in indicating that necrosis occurred according to Resnik.

Even Hughes (1969) cons dered more than ischemia to occur for S-GOT to be liberated from cardiac fibers and said that there must be necrosis with disintegration of cell walls.

Hendrich et al. (1970) however, pointed out that enzyme tests have not resolved the question of the clinical differentiation of angina pectoris as transitory myocardial ischemia from the more serious coronary attacks accompanied by myocardial necrosis. They showed a slight LDH -elevation in 20 per cent of thei angina pectoris patients and stated that this indicated that occasionally the angina gives rise to a certain degree of myocardial damage but assumed that it would not be correct to call this cond tion by any other name than angina.

Krauss et al. (1972) have discussed these patients who fall between stable angina and acute infarction and defined prolonged coronary pain without enzymatic and electrocardiographic evidence of AMI as acute coronary insufficiency

Several authors (Batsakis and Briere, 1967 Nozawa, 1970, Favaloro et al. 1971 Herman, 1971) Konttinen, 1971 have recently proposed a mild elevation f serum enzyme levels at the intermediate types of ischemic heart disease. Batsakis et al. (1967) said that in instances where modest rises are observed the source must be considered to be small and undetected areas of myocardial infarction and Herman (1971) stated that the underlying pathophysiology of the intermediate syndromes probably represents an acute change in the coronary circulation such as a subtotal occlusion of a vessel and that these conditions represent some degree of intramural or subendocardial tissue necrosis. Herman also pointed out the relationship between Prinzmetal s variant angina and intermediat coronary syndrome, supported by Poggi et al. (1971) who considered that "la crise d angor spontané de Prinzmetal se rapproche plus de la phase aiguë de l infarctus du myocarde que de l angor spontané classique"

Fowler (1971) stated that preinfarction usually is defined by clinical criteria which are in part subjective. By definition no diagnostic alterations are shown in the serum enzymes. This was supported by Scanlon et al. (1971)

There is in fact a source of controversy on this subject (Friedberg, 1972) *In the present study there is a distinction between angina of unstable type which is defined as Fulton et al (1972) and intermediate coronary syndrome which is defined as an episode of chest pain causing the patient to seek medical help and accompanied by an increase of S-GOT values within the normal range with more than 10 units within 24—48 hours from onset of symptoms* In this diagnostic group there were 2 cases of Prinzmetal's angina supporting the hypothesis that this condition sometimes is not an angina but a small infarction.

The reason for having an intermediate diagnostic group is a still insufficient laboratory and clinical accuracy in differentiation between myocardial ischemia and myocardial necrosis.

According to Friedberg (1972) a major trend in diagnosis is to obliterate the sharp boundaries between no infarct, subendocardial infarct and -mural infarction and to regard myocardial infarction as a continuous spectrum in terms of severity and extent of necrosis from involvement of microscopic areas to fullblown transmural infarction.

Wilkinson (1971) said in his review over the future of diagnostic enzymology that the findings of diagnostic enzymology will always have to be interpreted in relation to the relevant clinical and other data, and Batsakis and Briere in their Interpretive Enzymology (1967) stressed that a positive result should be regarded as confirmatory and not diagnostic.

Blörck and Hansson (1956) showed the relation between S-GOT positive AMI and the clinical and electrocardiographic picture and pointed out that additional methods for the diagnostic decision in questionable cases would be of definite value.

In the present study most of the patients subsequently diagnosed as AMI patients had typical enzyme curves. There were some patients with modified courses but in all these patients the frequent analyses with a combination of enzyme tests and ECG-changes were enough for establishing the diagnosis.

Several authors recommended a modified cardiac enzyme panel for investigation of chest pain (Hamolsky and Kaplan, 1961 Kibe and Nilsson, 1967 Coodley 1968 Reusch and Bunch 1968, Hughes, 1969 Albertini et al. 1970) There are great risks in judgement of single enzyme values. In the present study more than a fourth of the OBS-patients had atypical S-GOT-elevations.

Several reports are written about iatrogenic aberrations in this field (Batsakis et al 1967 Coodley 1968, Crowley 1968 Hughes et al. 1969 Popescu et al. 1969 Rowan, 1969 and Hammermeister and Merendino 1971 Sobel and Shell, 1972) The most common iatrogenic pseudo-myocardial syndrome is an opiate induced pressure increase in the biliary tract which may produce all of the symptoms and findings of myocardial infarction, in those in whom there is a biliary tract disease or in the absence of a gallbladder (Batsakis et al. 1967 Rowan, 1969) The S-GOT maxima in pseudo-myocardial infarct syndrome after administration of opiates are observed at five to eight hours after the administration (Batsakis et al. 1969) Iatrogenic elevation of CPK may occur with aqueous penicillin given intramuscularly four to five times the upper limits of normal (Rowan, 1969) Salicylates have been reported to elevate S-GOT (Hamolsky and Kaplan, 1961) Direct current countershock therapy in the treatment of cardiac arrhytmias may produce elevated CPK and S-GOT values (Sobel and Shell, 1972)

Crowley (1968) pointed out that CPK is known to be elevated following vigorous muscular activity and following epileptic convulsions. Hemolysis produced by prosthetic valves may be associated with slight elevations of LDH (Hammermeister and Merendino, 1971)

There are several conditions which can confuse the enzymatic picture (Goldberg and Winfield, 1972, Sobel and Shell, 1972) The slow release of enzymatic material from the injured cellular

stocks at acute myocarditis does not cause characteristic changes (Popescu et al 1969)

Increased enzyme activity in cardiac failure and in pulmonary embolism has been reported (Sobel and Shell, 1972) A normal CPK confirms the absence of cardiac muscle participation as a cause of the rise of other enzyme activities and the LDH isoenzyme fractionation shows increased prominence of the hepatic fractions (Batsakis et al. 1967)

Acute pectoral myositis gave false positive CPK in noncoronary patients as did hypothyroidism, other active muscle diseases or cerebral hemorrhage.

The frequent serum analyses in the present study have given an increased accuracy in diagnosing the patients admitted to the CCU and revealed a group of patients with very small enzyme elevations and without typical ECG-changes

All the patients in this group have had previous ischemic heart diseases in their past history (page 35) supporting the final diagnosis of a coronary syndrome in these patients.

SUMMARY

Frequent serum enzyme determinations during the first 4 hours in the CCU have been done in 191 patients.

In 46 per cent the enzyme curves fulfilled the diagnostic criteria for acute myocardial infarction (AMI) and in 44 per cent this diagnosis could be excluded (OBS)

In the remaining 10 per cent the diagnosis was intermediate coronary syndrome (ICS) defined as an episode of chest pain causing the patient to seek medical help accompanied by an increase of S-GOT-values within the normal range but with more than 10 U/l within 24—48 hours from onset of symptoms and without typical ECG-changes suggestive of an AMI.

The accuracy of the diagnosis of ICS is discussed and a review over iatrogenic aberrations and clinical conditions, which can confuse the enzymatic picture is made.

The Early Clinical Picture of Patients Admitted to a CCU

— a prospective study of 191 patients —

It is apparent from the study presented in part I that there are some differences between the patients admitted to the CCU on suspicion of having acute myocardial infarction whether this subsequently is verified or not.

Any convincing separation between the two diagnostic groups—AMI och OBS-patients—was not obtained with the discriminant function analyses presented in part II and based on the information in part I.

In an attempt to further improve the diagnostic accuracy in the early stage of acute myocardial infarction, a prospective study of consecutive patients admitted to CCU was performed.

The aims of the present study were:

1. to analyze all the patients systematically with a detailed questionaire regarding previous history symptoms before onset of the attack leading to the hospitalisation as well as the symptoms of the acute attack (History and symptoms)
2. to collect similarly simple clinical parameters on admission to CCU like the blood pressure, the pulse pressure, the respiratory rate the heart rate and the occurrence of pulmonary rales (Signs)
3. to perform more frequent enzyme analyses than the traditional routine allowed (Tests) (Part III page 26)
4. and to bring all this information together in order to improve the diagnosis of AMI in its early stages.

MATERIAL AND METHODS

The series consisted of 236 consecutive admissions to the CCU Serafimerlasarettet from February 15 1971 to July 1 1971 These 236 admissions corresponded to 191 individual patients If a patient has been treated in the CCU as an OBS-patient within one month before an admission because of an AMI he has been classified as an AMI patient and the previous episode has been judged as a prodromal symptom. A patient admitted to the CCU because of new symptoms within one month after an AMI without verification of a new myocardial infarction has also been classified as an AMI patient and the new admission interpreted as a status post myocardial infarction (Verghese and Lovell, 1966) Patients admitted more than once with the diagnosis of Observation have been classified as one OBS-patient, and the first episode has been the one included in this study

The ordinary routine of the CCU

The organization and the ordinary routine of the CCU has been described in part I

The history taking
The questionaire

A questionaire was constructed to cover systematically prodromal symptoms and acute symptoms and events leading to hospitalisation. Furthermore the history of previous diseases was included. The questionaire was the basis for a personal interview by the author

Interviews

During the day the patients were mostly interviewed in direct connection with the admission to the CCU Patients admitted 19.00—07.00 were interviewed the morning following admission. In most of the cases neither the patient nor the interviewer had any information as to whether the symptoms were caused by an AMI or not. In some cases, however the interviewer who during the

study was on duty in the CCU knew the diagnosis because of ECG signs suggestive of AMI on admission. As far as was possible the patients were kept uninformed about this until the end of the interview. The interviews generally took about 20 minutes and usually the patients themselves gave all relevant information. In some cases a relative of the patient supplemented the information.

Addendum. A retrospective study of the hospital records of all patients treated in our hospital during the same time with the diagnosis of AMI revealed 6 patients with AMI who have not been admitted to the CCU. Three men—one with a cerebrovascular accident, one with pulmonary oedema and one who was unconscious at the arrival to the Casualty Department and who died within one hour—and 3 women—all of advanced age with complex symptoms on admission. During the same time 2 other men were treated in the hospital with the diagnosis of suspected AMI because of small elevations of the enzymes.

Physical examination at admission

Systolic and diastolic blood pressure, pulse pressure, heart rate, respiratory rate and occurrence of rales or fulminating pulmonary oedema on admission to the CCU were noted in the chart of every patient. When the author was not the first physician to examine a patient this information was obtained from the ordinary registration charts described in part I.

Electrocardiography

The same method in recording the ECG described in part I was used (Page 12).

Serum enzyme determinations

The enzyme determinations used in this study are described in part III (Page 26).

DEFINITIONS

Angina pectoris, previous myocardial infarction, hypertension and heart failure are defined as described in part I (Page 13).

Diabetes mellitus. A history of diabetes mellitus treated with antidiabetic agents.

Other diseases. The patient's own information of previous diseases was accepted.

Statistics. The same principles as in part I concerning significance of differences between mean values and of relative numbers (Page 13).

DIAGNOSTIC CRITERIA

Diagnosis based on electrocardiography

The same electrocardiographic criteria for the diagnosis of AMI outlined in part I were used (Page 13).

Diagnosis based on enzyme determinations

The enzymatic criteria of acute myocardial infarction are discussed and presented in part III. Enzyme diagnosis in symptoms leading the patient to a coronary care unit. (Page 26).

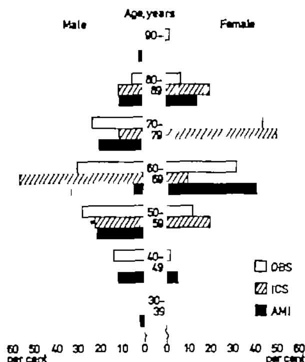
RESULTS

Acute myocardial infarction (AMI) was diagnosed in 88 patients (46 per cent) according to the criteria part III, and in 19 patients (10 per cent) the diagnosis was Intermediate Coronary Syndrom (ICS) also according to the enzyme result presented in part III. The remaining 84 patients (44 per cent) had no enzyme findings which could possibly be the basis for a diagnosis of AMI (OBS).

Sex and age distribution

Sex. In the AMI group there were 66 men (75 per cent) and 22 women (25 per cent) giving a sex ratio of 3:1. In the ICS-group there were 9 men and 10 women giving a sex ratio of 1:1 which is similar to that in the OBS-group where there were 43 men and 41 women.

Age. Fig. 6 shows graphically the age distribution for men and women in the present study. The mean age for AMI-patients was 65.6 years—64.1 for men and 70.4 for women. The ICS-patients had a mean age of 68.7 years—65.6 for men and 71.5 for women. The difference between



F g 6. Age distribution in relation to sex in the three diagnostic groups of patients.

the AMI and ICS-patients was not statistically significant. In the OBS-group the mean age was about was about the same as in the AMI group 65 7 years—62.1 for men and 69 5 for women.

Comments

In this study there were slightly more men than women in the AMI group compared to the corresponding group of patients described in part I The mean age of the men in all three diagnostic groups was lower than the mean age of the corresponding women. It was higher in the ICS-group than in the two other groups. One man in the AMI group was below 40 years of age and this man was from Turkey One Swedish woman in the AMI group was below 50 years of age. The AMI-diagnosis in men below 40 and women below 50 years of age is extremely uncommon in Sweden (Biörck 1960 a, Wikland 1971 Ahlmark and Gillgren 1972) The age distribution corresponds with that presented in part I

A PAST HISTORY

First there will be presented an analysis of the occurrence of diseases traditionally associated with AMI as angina pectoris, previous myocardial infarction, hypertension, heart failure and diabetes mellitus. Other diseases in the past history are described under the heading of Co-morbidity

Previous angina pectoris

Angina pectoris with a duration of less than 1 month prior to admission has been classified as prodromal symptom and not counted here. A history of angina pectoris was given by 47 per cent of the AMI patients and by 58 per cent of the OBS-patients. Only 1 patient in the ICS-group was without history of angina pectoris (Table 16)

Angina pectoris with a duration of less than 6 months prior to admission occurred in 9 per cent of the AMI patients against 1 per cent of the OBS-patients while none of the patients in the

TABLE 16. The incidenc and duration of previous angina pectoris in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 2 per ent	Total = 88 per ent	Males = 9 per cent	Females = 10 per ent	Total = 19 per cent	Males = 43 per cent	Females n = 41 per cent	Total = 84 per cent
N angina	5	53	53	11	–	5	11	39	42
<6 m	11	5	9	—	–	—		—	1
6 m—2 yrs	6	5	6	11	–	5	5	5	5
yrs—10 yrs	15	3	1	67	40	53	28	7	27
>10 yrs	15	11	15	11	60	37	21	79	25

ICS-group mentioned angina pectoris of short duration. The difference between AMI and ICS-patients was highly significant and significant between the OBS- and ICS-patients

Six per cent of the AMI patients reported on angina pectoris with a duration of more than 6 months but less than 2 years. Angina pectoris of this duration was indicated by 5 per cent in each of the other two groups of patients

Angina pectoris with a duration of more than ten years occurred in 15 per cent of the AMI patients against 25 per cent of the OBS-patients while more than a third of the ICS-patients had a history of angina pectoris lasting for more than 10 years. The difference between AMI and ICS-patients was almost significant ($p < 0.05$)

Thirty eight per cent of the AMI patients used nitroglycerine against 56 per cent of the OBS-patients. Nearly all the patients in the ICS-group used nitroglycerine (90 per cent)

Comment

The incidence of angina pectoris was about the same in the AMI and OBS-groups, while it was considerably higher in the ICS-group

The incidence of angina pectoris in patients with AMI is reported to be about 45—50 per cent in several Swedish investigations on AMI (Biörck, 1962, Bevegård et al. 1970 Bronson and Malmcrona, 1971 Henning and Holmberg, 1971) In studies from other countries the incidence varies from 40—65 per cent in different AMI-materials. (Billings et al. 1949 Chambers, 1946, Moss, 1964, Lown, 1968 and Bailey et al 1968)

The occurrence of angina pectoris in patients admitted to the CCU without a subsequent verification of AMI is difficult to estimate from the literature. Engstedt et al. (1970) noted 34 per cent in their OBS-group and in Hofvendahl's series (1970) 50 per cent gave a history of angina pectoris and/or myocardial infarction.

The problems with unstable angina pectoris, prodromal angina and pre-infarction-angina are discussed under the heading of Prodromes (page 39)

Previous myocardial infarction

Table 17 shows the incidence of previous myocardial infarction in the three diagnostic groups. Thirty two patients (36 per cent) in the AMI group gave history of previous myocardial infarction and 32 patients (38 per cent) in the OBS-group while 11 (58 per cent) of the 19 ICS-patients had a previous myocardial infarction in their past history None of these differences were significant.

Comments

With the introduction of the CCUs the hospital mortality of acute myocardial infarction has been reduced (Hofvendahl 1971 and Christiansen et al. 1971) This fact ought to lead to an increase of the prevalence of previous myocardial infarction in patients admitted to the CCU In this study the incidence is rather high but corresponds to the findings in part I. In other Swedish studies the incidences varies from 17 per cent to 31 per cent

TABLE 17 The incidence of previous angina pectoris (AP) and myocardial infarction (MI) in the three diagnostic groups and the incidence of no previous IHD-manifestation in the same groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Previous AP	47	47	47	89	100	95	56	61	58
Previous MI	36	36	36	44	70	58	44	32	38
No previous IHD	41	46	42	0	0	0	40	34	37

(Bevegård et al 1970 Henning and Holmberg, 1971 and Brorson and Malmcrona 1971) One reason of the high incidence of previous myocardial infarction is perhaps that there is no upper age limit in our admission criteria.

No previous IHD manifestations

Previous IHD-manifestations were defined as occurrence of angina pectoris and/or myocardial infarction in the past. Thirty seven patients (42 per cent) in the AMI-group were without history of previous IHD and 31 (37 per cent) in the OBS-group while none of the ICS-patients were without IHD (Table 17)

Comments

The number of patients without previous manifestations of IHD in the AMI group is in accordance to the findings in part I (page 14) and to other reports (Bröck 1962 Lown 1967 Wikland 1971) The similarity in this respect between the AMI and OBS-patients is interesting.

Remarkably all the patients in the ICS-group had a history of angina pectoris and/or myocardial infarction. Only 31 patients (16 per cent) were without manifestations of IHD at the time of this investigation but the immediate constellation of symptoms in these patients does not exclude the onset of IHD

Hypertension heart failure and diabetes mellitus

The incidence of hypertension was 19 per cent in the AMI group 21 per cent in the ICS-group and 18 per cent in the OBS-group (Table 18)

Previous heart failure was reported by 36 per cent of the AMI patients, 68 per cent of the ICS-patients and 54 per cent of the OBS-patients (Table 18) The differences between the AMI patients and the two other groups were almost significant ($p < 0.05$)

In Table 18 it is shown that 7 per cent of the AMI patients had diabetes mellitus before admission—3 per cent of the men and 18 per cent of the women. In the ICS-group 4 patients had diabetes mellitus—1 man and 3 women. The incidence of diabetes in the OBS-group was 5 per cent—2 per cent in the men and 7 per cent in the women

Comments

The incidence of hypertension ranges from 14 to 74 per cent in the literature (Thomas et al 1968 and Chambers, 1946) Some authors have pointed out that hypertension is more common in women (Billings et al. 1949 Maurice et al. 1954) In this study there was no statistical difference between the sexes and no differences between the three diagnostic groups.

The incidence of previous heart failure was about the same in the AMI patients as in the corresponding patients in part I In this study there was an almost significant difference between the AMI patients and the two other groups. The incidence of heart failure was high in the ICS-group

In the literature the incidence of heart failure in the past history of patients with acute myocardial infarctions differs from 11 per cent (Chambers, 1946) to 42 per cent (Brorson et al. 1971)

TABLE 18 The incidence of previous hypertension, heart failure and diabetes mellitus in the three diagnostic groups

	AMI			ICS			OBS		
	Males 66	Females 2	Total = 88	Males = 9	Females = 10	Total = 19	Males = 43	Females = 41	Total = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Hypertension	15	3	19	22	20	21	7	29	18
Heart failure	30	55	36	6	70	68	17	61	51
Diabetes	3	18		11	30	21	2	7	5

Concerning diabetes mellitus the incidence of this disease was low in the men in the AMI group while the incidence in the women corresponds to that found in the literature.

Diabetes mellitus occurred in 8—17 per cent in different series of AMI patients (Chamber 1946, Billings et al., 1949 Plotz, 1957 Wahlberg, 1963 and Sievers, 1964) The incidence is commonly higher in women than in men (Sievers, 1964, Bailey and Bearven, 1968) which was confirmed in this study as well as in part I

The occurrence of diabetes mellitus was high in the group of patients with slight elevations of the enzyme values after the onset of symptoms (The ICS-group)

Co morbidity

Any review over the co-morbidity in patients with IHD has not been found in the literature. The occurrence of different diseases in the patients of this study is presented Table 19

Peptic ulcer

Eighteen AMI patients (21 per cent) gave history of this disease. The same incidence was indicated in the ICS-group and in the OBS-group. 13 patients (16 per cent) gave a positive answer in this respect.

The incidence of this condition was higher in the men in the AMI and ICS-group than in the corresponding women ($p < 0.05$) but about the same in both sexes in the OBS-group (Table 19)

Renal calculus

The incidence of a renal calculus was high in all diagnostic groups (10—16 per cent) However there were no differences between the groups.

Gout

Only a few of the patients gave a history of gout. One patient in the AMI group, 4 in the OBS-group and none in the ICS-group gave a history of this disease.

Diseases of the respiratory system

Asthma bronchiale, chronic bronchitis, emphysema or healed pulmonary tuberculosis have been classified as chronic respiratory diseases of importance in the past history. History of acute pneumonias, common cold and other more temporary diseases has not been evaluated in this study

A history of chronic respiratory diseases was positive in 11 AMI patients (13 per cent) in 1 ICS-patient (5 per cent) and in 7 OBS-patients (8 per cent)

Diseases of the urinary tract

Pyelonephritis or cystopyelitis occurred in 4 AMI patients and in 5 OBS-patients.

TABLE 19. The co-morbidity in the three diagnostic groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Peptic ulcer	24	9	21	44	—	21	16	15	16
Renal calculus	18	5	14	22	10	16	9	10	10
Gout	2	—	1	—	—	—	7	—	3
Resp disease	15	5	13	—	10	5	2	15	8
Pyelonephritis	6	—	5	—	—	—	9	2	6
Hepatic and biliary disease	11	46	22	—	30	16	12	2[illegible]	17
Goiter	3	9	5	—	—	—	5	2	4
Malignancy	—	5	1	—	—	—	—	2	4
Cerebrovasc disease	2	9	3	—	—	—	2	7	5
Other	3	9	6	—	10	5	9	12	11

Hepatic and bilary disease

In 19 AMI patients (22 per cent) in 3 ICS-patients (16 per cent) and 14 OBS-patients (17 per cent) there was a positive history of disease of the liver or the gall bladder mainly in form of cholecystitis with or without stones. The incidence was higher in women in the AMI and ICS-group than in the corresponding men ($p<0.001$) but about the same in both sexes in the OBS-group

Goiter

Four AMI patients and 3 OBS-patients gave a positive history of diseases in the thyroid gland.

Malignancy

One AMI patient had been operated because of a neoplasm of the rectum, while four OBS-patient had a history of malignancy myelomatosis, reticelcell sarcoma, neoplasm of the sigmoideum and in the urinary bladder

Cerebral lesions

Four AMI patients end four OBS-patients had had cerebrovascular accidents with different degrees of remaining symptoms.

Comments

The incidence of peptic ulcer in patients with coronary disease varies in the literature. Sievers (1964) showed that 71 patients in his study had had peptic ulcer—9 per cent in men above 60 years and 6 per cent below 60 and 3 respectively 1 per cent in the correspond ng ages n the women Watkinson (1956) who based his conclusions on autopsies, said that there was a significant relation between coronary sclerosis and ulcers in men, especially in younger ages.

In the present series the incidence was rather high. One explanation may be that a systematical questioning of the patients gives a higher incidence than retrospect ve studies of hospital records. The incidence of these complaints was higher for men than for women the AMI and ICS-group

Ask Upmark and Adner (1950) pointed to an association between gout and coronary heart disease. The result of th tud do ot ont rm this observation, but the present series may be too small in this respect.

Thomas et al. (1968) found chronic bronchitis in 10 per cent of their AMI patients.

Concerning bile stone, Johnsson (1959) has given a incidence of 2 per cent and Billings et al. (1954) 13 per cent. Forrester et al. (1970) showed that the incidence of gall bladder disease is not greater in patients with than without coronary heart disease.

In the present study the incidence was higher in the women in the AMI and ICS-group compared to the men in these groups, while the incidence was about the same in both sexes in the OBS-group.

Other diseases analyzed in the present study were represented in about the same incidences in all three diagnostic groups.

To sum up the co-morbidity the following can be concluded Peptic ulcer renal calculus and gall ston- were common previous cond tions in the patients admitted to the CCU There were no particular differences between patients subsequently diagnosed as AMI patients, ICS-patients or OBS-patients. Other diseases were more uncommon and with unsystematic occurrence in the three diagnostic groups of patients.

Smoking Habits

The smoking habits of the patients in this study are shown n Table 20. About half of the OBS-patients and half of the ICS-patients had never smoked against only 19 per cent of the AMI patients ($p<0.001$)

A fourth to a third of the patients in all three diagnostic groups were former smokers.

Fourty per cent of the AMI patients were cigarette smokers against 11 per cent of the ICS-patients and 23 per cent of the OBS-patients.

Cigar and pipe smokers were represented in 14 per cent of the AMI group 11 per cent in the ICS-group and 4 per cent in the OBS-group.

As can be seen in Table 20 the smoking habits aried between the sexes but the d fferences betw en the diagnostic groups are still sign ficant if the x are studied separately

Comments

The incidence of a positive history of smoking was higher in the AMI group than in the two other groups.

This difference is interesting as nearly all patients in the present study have previous IHD manifestations and the smoking habits would be expected to be nearly the same in the three different groups.

Gorbatow (1961) noted that 71 per cent of the AMI patients were smokers against 55 per cent in a control group. He also pointed to the different smoking habits in men and women. Engstedt et al. (1971) showed an incidence of 40 per cent in the AMI patients and 41 per cent of their OBS-patients. Henning and Holmberg (1971) found 70 per cent in their study of AMI-patients.

To sum up, the incidence of smokers or former smokers was higher in the AMI group than in the two other groups. This in spite of a high incidence of IHD manifestations even in these two latter groups.

B PRODROMES

Increased knowledge about the course of the AMI with awareness of the high initial mortality has caused many authors to focus interest on the time before the onset of the acute event order to discover prodromal symptoms

Solomon et al (1969) maintained that 65 per cent of the patients their study had prodromes of some kind Renggli (1971) related an incidence of 70 per cent and Hochberg (1971) not less than 84 per cent.

In the older literature the incidence of prodromes aries greatly and Mounsey (1951) who in his series indicated an incidence of 29 per cent, pointed out that the discrepancy between different studies could depend on different compositions of the materials and on different opinions about what constitutes prodromes.

The importance of continuing studies of this subject has recently been emphasized by a WHO Working Group on the Prodromal Symptoms of Myocardial Infarction and Sudden death (1971)

The time defined as prodromal time differs. Solomon (1969) considered two months as reasonable and Mounsey (1951) three months. Renggli (1971) in her study estimated one month as the period of prodromes.

In the present study prodromes have been defined as symptoms started or aggravated within one month before admission to the CCU

Methods

The 191 patients in the present study were systematically asked about occurrence of new or increasing symptoms appearing during the last month.

By asking the patient if the acute attack has started like a bolt from the blue, the patient usually understood the purpose of the question.

TABLE 70 Smoking habits in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Non smokers	12	41	19**	11	80	46	25	75	47**
Former smokers	3	14	77	56	10	32	33	15	24
Cigarette smokers	58	45	40	11	10	11	35	10	23
more than 30-day	3	5	3	—	—	—	5	—	2
Pipe-Cigar smokers	18	—	14	2	—	11	7	—	4

p < 0 5 ** p < 0 001

The patient was asked if he during the last month before admission had noted any new or uncommon symptoms of some kind. After that he was systematically asked about the following symptoms in layman terms

Onset of angina pectoris
Crescending angina pectoris
Recent onset of atypical pains in chest or arms
Onset or worsening of dyspnoea
Onset of dizziness
Onset of progressiv or new weakness or fatigue
Onset of new symptoms from the gastrointestinal organs in form of flatulence, obstipation or diarrhoea
Loss of appetite
Onset of profuse sweating
Onset of arrhythmic sensations

The prodromal time was noted for each patient.

The patients were also asked if they had visited a physician during the last 3 months either for a regular check up or on account of new symptoms.

Results

There was some change of the symptomatology in 68 per cent of the AMI patients within one month before admission to CCU (Table 21). The patients in the ICS-group reported prodromes in 53 per cent while 46 per cent of the OBS-patients had noticed onset or changing of symptoms during the month before the attack leading the patient to the hospital. The difference between AMI and OBS-patient was highly significant ($p < 0.001$)

Pain as a prodrome

The most common prodrome before admission was new or increasing chest pain. This was reported by 55 per cent of the AMI patients 47 per cent of ICS-patients and 24 per cent of the OBS-patients. The difference between the AMI and the OBS-patients was highly significant ($p < 0.001$)

Atypical pain as a prodrome

Onset of atypical pain in chest or arms was reported by 16 per cent of the AMI-patients. Some patients described a discomfort in the chest in the form of a pricking sensation, a traction in the right side, a stitch or a spasm. Some patients had had an onset of pain in the right or left arm. These uncharacteristic descriptions occurred also in 11 per cent of the ICS-patients and in 16 per cent of the OBS-patients.

TABLE 21 Prodromata. A comparison of new or crescending symptoms one month before admission to CCU between the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total n = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 81 per cent
Prodromes of any kind	71	59	68	56	50	53	42	51	46
Chest pain	56	50	55	56	40	47	26	22	4
Dyspnoea	4	18	23	—	—	—	21	27	1
Tiredness	77	36	30	11	70	16	12	44	7
Autonomic symptoms	15	5	13	—	20	11	9	17	13
Loss of appetite	11	9	10	11	10	11	12	20	16
Arrhythmic sensations	6	23	10	22	—	11	16	15	16
Flatulence, diarrhoea	11	27	15	11	10	11	23	10	17
Vertigo dizziness	6	—	5	11	30	1	9	79	19*
Headache	3	—		—	10	5	5	5	5
Miscellaneous descriptions	1	14	16	—	20	11	14	17	16

$p < 0.01$ $p < 0.001$

Dyspnoea as a prodrome

Appearance or worsening of difficulties in breathing was reported by 23 per cent of the AMI patients and 24 per cent of the OBS-patients while none of the patients in the ICS-group had noticed any changes in this respect.

Weakness or fatigue as a prodrome

Onset of an unnatural weakness or fatigue was mentioned by 30 per cent of the AMI-patients 16 per cent of the ICS-patients and 27 per cent of the OBS-patients.

Other symptoms as prodromes

Onset or worsening of arrhythmic sensations during the month before admission was found in 10 per cent of the AMI patients and in 11 per cent of the ICS-patients while 16 per cent of the OBS-patients had noticed this.

As can be seen in Table 21 the incidence of other symptoms varies and was more uncommon. Onset of vertigo, dizziness, sensation of light headness was indicated by 5 per cent of the AMI patients while 21 per cent of the ICS-patients and 19 per cent of the OBS-patients have had experiences of this kind.

Loss of appetite was reported by 10 per cent of the AMI-patients, 11 per cent of the ICS-patients and 16 per cent of the OBS-patients

Autonomic symptoms, usually sweating, had started in 13 per cent of the AMI patients, in 11 per cent of the ICS-patients and in 13 per cent of the OBS-patients.

Two per cent of the AMI patients had got headache of a kind they previously not experienced the last month before admission, so had 5 per cent of the ICS-patients and 5 per cent of the OBS-patients.

Disturbance of gastrointestinal functions in form of onset of flatulence diarrhoea or obstipation was reported by 15 per cent of the AMI-patients, 11 per cent of the ICS-patients and 17 per cent of the OBS-patients.

The prodromal time

In 7 per cent of the AMI patients the prodromes began the day before admission. The symptoms thus defined as prodromes were not the same immediate symptoms leading the patients to the hospital. Eleven per cent of the ICS-patients and 4 per cent of the OBS-patients also reported prodromes the day before onset of the immediate attack.

Prodromes with onset within one week before admission were found in 30 per cent of the AMI patients, in 16 per cent of the ICS-patients and in 23 per cent of the OBS-patients.

A prodromal time of a forthnight was reported by 12 per cent of the AMI patients, 5 per cent of the ICS-patients and 7 per cent of the OBS-patients.

Prodromes with a duration of more than fourteen days, but starting within a month before admission occurred in 26 per cent of the patients in the AMI group and in 32 per cent of the ICS-group while 17 per cent of the OBS-group mentioned prodromes of that length. (Table 22)

TABLE 22 Prodromal time in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
No prodromes	29	41	32	41	50	47	58	51	54
1 month	29	18	26	33	30	32	9	24	17
2 weeks	15	5	12	11	—	5	2	12	7
1 week	20	32	23	11	—	5	23	12	19
1 day	8	5	7	—	20	11	5	2	4

Visiting a physician before admission

To clarify how the patients act on prodromes they were asked if they had visited a physician during the last months before admission, either because of a routine check up or because of new symptoms.

Regular contact with a physician because of chronic disease was reported by 25 per cent of the AMI patients, by 32 per cent of the ICS-patients and by 38 per cent of the OBS-patients.

A further 34 per cent of the AMI patients had seen a physician within the last month before admission because of onset of new symptoms and in half of these cases they had seen the physician within the last week. In the ICS-group the figures were about the same—32 per cent and 16 per cent. Twenty nine per cent of the OBS-patients had also seen a physician the month before admission because of new symptoms and a third of these in the last week.

Thirty two per cent of the AMI patients were without any contact with a physician the last three months before admission which was also the fact in 25 per cent of the ICS-patients and in 2 per cent of the OBS-patients. (Table 23)

Discussion

There are considerable difficulties inherent into an evaluation of the occurrence of prodromes in patients admitted to hospital with an acute attack of chest pain. Retrospective studies can give false interpretations of atypical symptoms. Hindsight may affect the patient's description of his symptoms as well as the doctor's interpretation of them (Fulton et al 1972).

A patients knowing himself as being in the initial phase of an AMI may overemphasize symptoms like tiredness and even progressive chest pain before onset of the immediate attack. Furthermore, studies do not reveal how often such symptoms do not end with an AMI.

In this study the patients were interviewed very early after the onset of symptoms which had brought them to hospital. Generally the patients were not aware if the symptoms had been caused by an AMI or by something else. However all patients knew of the suspicion of an AMI as they had been admitted to a CCU and thus hindsight ought to play the same role in all these diagnostic groups.

The incidence of prodromes of some kind was high in all three diagnostic groups but the difference between the AMI and OBS-patients was highly significant. This difference was mainly caused by the development of a new or increasing chest pain. Other symptoms were of less discriminative importance as the incidence was about the same in the three groups. Dyspnoea, fatigue, atypical pain, sweating and loss of appetite occurred equally often in the three groups. Many of these symptoms are common in the general population and may precede other disease processes.

The number of patients visiting a physician within a month before admission was high in all three groups. This is in accordance with several authors (Kuller et al. 1966, Stowers et al. 1970, Wikland 1971, Fulton et al. 1972).

One third of the AMI patients had visited a doctor during the preceding month but the same proportion of the OBS-patients had also done so.

TABLE 23 The incidence of contact with physician before admission to CCU in the three diagnostic groups

	AMI			ICS			OBS		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
	[illegible]	[illegible]	= [illegible]	= 9	= 10	= 19	n = 43	= 41	= 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Routine check up			[illegible]	[illegible]	[illegible]	3	4	31	35
Acute [illegible] the last 3 months				1	1	11	1	10	11
Acute [illegible] the last month		I		11	[illegible]	16	14	[illegible]	19
Acutely the last week						16		12	10

This reveals that the early judgement of these patients is very difficult. Also about a third of the ICS-patients had seen a doctor within the last month. There were several reasons for seeing a doctor: for instance atypical chest pain, common cold, abdominal pain and routine check up. Only a 25 to 30 per cent of all the patients were without medical control the time before admission.

Mounsey (1951) indicated premonitory symptoms in 29 per cent of his AMI patients. Maurice et al. (1954) reported 40 per cent and Steifa and colleagues (1962) 45 per cent in their study. In other studies (Solomon et al. 1969, Moss 1970, Stowers et al. 1970, Renggli 1971, Skjaeggestad 1971, Furberg et al. 1972) the incidence varies from 50 to 70 per cent. Only a few authors have given information about prodromes in patients without AMI. In Solomon's investigation (1969) none of the 25 control patients gave a history of prodromes. Ander et al. (1971) found chest pain before admission in 76 per cent of the cases with AMI and only in 12 per cent in a screening group of patients. Hochberg (1971) pointed out that the occurrence of prodromes was high even in a group of patients with what he called acute coronary insufficiency without AMI. His figures were 93 per cent in a group of patients with acute coronary insufficiency compared with 84 per cent in an AMI group.

Prodromes occurred several patients in the present study who did not develop myocardial infarction. The incidence was lower than in Hochberg's study but the OBS-patients were not only patients with so called acute coronary insufficiency. Further prospective studies must be done to reveal how often chest pain, considered to suggest premonitory pain, would represent a false alarm (Friedberg, 1972).

Fulton et al. (1972) in their report on the natural history of unstable angina had noticed occurrence of preceding unstable angina in 60 per cent f the 110 cases with myocardial infarction their study.

T sum p the occurrence of new or changing symptoms preceding the admission to the CCU is f discriminating power between the AMI and OBS-patients in this study in spite of the rather high incident of prodromata even in the OBS-patients.

New or increasing chest pain, unstable angina (Fulton et al. 1972) was especially discriminating between the AMI and the OBS-group. This is also in accordance with the report of the WHO Working Group on the Prodromal Symptoms of Myocardial Infarction (1971) who expressed that only changing patterns of chest pain appear to be especially associated with acute heart attacks and therefore can be regarded as truly premonitory.

C THE ACUTE ATTACK

The day of onset

Several authors have studied the occurrency of AMI in relation to the days of week (Master 1937 b, Strbder et al. 1951, Berg et al. 1957, Jensen, 1959, Gorbatow et al. 1962, Lindholm, 1963, Askansas et al. 1970).

In the present study there was a higher morbidity during Saturday, Sunday and Monday in the AMI group than in the two other groups of patients. In 56 per cent of the AMI patients the onset of symptoms started during one of these three days against 37 per cent in the ICS-group and 42 per cent in the OBS-group. The difference in this respect between the AMI and the OBS-patients was almost significant ($p < 0.05$) (Table 24).

The lowest rate in the AMI group was noted in Tuesdays but there were no statistical differences between the three groups.

Comments

According to some authors (Berg et al. 1957, Jensen, 1959, Uhlenbruck, 1967) the risk is greatest during the weekends owing to parties or other entertainment on Saturdays, a heavy rich meal on Sundays, usually with alcohol and often very strenuous work on Mondays (Lindholm, 1963). In this study there was also a difference between the AMI patients and the OBS-patients in this respect. In one study recently published from Warsaw (Askansas et al. 1970) there was a significant trend of decrease in the AMI incidence rate starting with the first to the last day of the week. (Mon-

TABLE 24 The day of onset of symptoms leading the patients to CCU A comparison between the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per ent	Total = 88 per cent	Males = 9 per ent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total n = 84 per ent
Sunday	18	23	19	—	10	5	7	24	16
Monday	20	18	19	11	20	16	12	7	10
Tuesday	9	5	8	22	10	16	19	17	18
Wednesday	14	5	11	33	10	21	9	10	10
Thursday	11	14	11	11	20	16	14	20	17
Friday	8	14	9	11	10	11	9	12	11
Saturday	15	23	17	11	20	16	23	7	16

day to Sunday) Even Master (1937 b) found a high frequency of onset of symptoms on Mondays while Gorbatow et al. (1962) and Lindholm (1963) found no statistically significant variation with the days of the week and no tendency for disease to occur during weekends.

In this study there was a sl ghtly higher incidence of onset of symptoms during the weekends than during the other days of the week in the AMI group but not in the other two groups of patients.

The time of onset

For the present study the day was divided into four parts shown in Table 25 There are no statistical differences between the groups. Eight per cent of the AMI patients and 11 per cent of the OBS-patients could not gi e any information about the time of onset.

Comments

There were no s gnificant differences between the groups concerning the time of onset in this study This was in accordance with the findings presented in part I (page 16) Some authors have shown a higher incidence in daytime (from 6 am. to 6 pm) than in the night (from 6 pm to 6 am) (Ströder et al 1931 Askansas et al. 1970) while others have pointed out this tendency but also emphazised that several patients cannot give the hour of onset and that most of these cases might have occurred during night (Lindholm, 1963)

The time between onset and admission to CCU

The time between onset of symptoms and admission to CCU i.e. the delay has been subjected to considerable nterest in relation to CCUs (Hack ett and Cassem 1969 Moss 1969 Gilchrist 1971

TABLE 25 Time of onset of symptoms in the three diagnostic groups

	AMI			ICS			OBS		
	Males 66 per ent	Females — per ent	Total = 88 per ent	Males = 9 per ent	Females = 10 per ent	Total = 19 pe ent	M les = 43 per ent	Females = 41 per cent	Total = 84 per ent
01—06	18	36	3	11	20	16	16	22	19
0 —1		18	3	44	30	37	33	29	31
13—18	3	14	2		30	6	3	10	17
19—00	18		24		20	1	19	7	3

Armstrong et al. 1972, Kamaryt et al. 1972 Tjoe and Luria 1972 Yu 1972) The delay varies in different communities probably due to differences in the medical knowledge of the public, availability of transportation and distances to the hospital from the place of occurrence of the attack as well as other factors. An important proportion of this time is called the patient delay which is that part which is influenced by the patient's own action.

In this study as can be seen in Table 26 delay of less than 3 hours is roughly equally common in all groups, while a delay of less than 6 hours was almost significant more common in the AMI and ICS-patients than in the OBS-patients ($p < 0.05$)

More than 75 per cent of all the patients were admitted within 12 hours with similar distribution in all three groups. Eight per cent of the AMI patients and 11 per cent of the OBS-patients could not give certain information about time of onset or delay

Comments

A delay of less than 6 hours was more common in the AMI and ICS-groups than in the OBS-group. This was in accordance to the finding presented in part I (page 16) One study presented by Engstedt et al. (1971) showed that the AMI and OBS-patients, were hospitalized in about the same frequency when calculating with a delay of less than 4 hours.

Tjoe and Luria (1972) have showed that there was no statistical difference in decision times of patients with chest pain admitted to the CCU whether or not their eventual diagnosis was that of AMI.

Wikland (1971) showed, in his study that there was no striking over or underrepresentation of previous myocardial infarction, angina pectoris, heart failure, hypertension or other disorders in the group of patients who call for medical assistance during the last fatal attack of chest pain in comparison to those who did not. In order to analyze the patient delay factors a more detailed investigation is being carried out in our CCU (Erhardt et al.)

Activity at onset of symptoms

Many authors have studied the physical and mental activity at onset of symptoms in acute myocardial infarction (Master, 1937 Blumgart 1945 Billings, 1949 Yater et al. 1951 Sievers, 1964, Wikland, 1971 Robert, 1972)

The recorded activities in the patients of this study are shown in Table 27

In the AMI-group 19 per cent of the patients were asleep at onset of symptoms and awaked with or by the symptoms. Eleven per cent of the ICS-patients and 16 per cent of the OBS-patients gave the same history

Activities such as lying down but not sleeping, sitting and standing still were defined as at rest and reported by 14 per cent of the AMI patients,

TABLE 26 Time between onset of symptoms and admission to CCU in the three groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
< 1 hrs	12	5	10	[illegible]2	70	1	9	2	6
< 3 hrs	23	18	2[illegible]	33	30	32	16	12	14
< 6 hrs	65	64	65	67	70	68	49	54	51
< 12 hrs	79	82	80	89	70	79	74	76	75
> [illegible]1 hrs	91	96	92	100	100	100	91	85	89

$p < 0.05$

TABLE 27 Types of activity at onset of symptoms on th patients in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 66 per ent	Females n = 2 per ent	Total n = 88 per ent	Males n = 9 per ent	Females = 10 per cent	Total n = 19 per ent	Mal = 43 per cent	Females n = 41 per ent	Total n = 81 per ent
Sleep	1	27	19	—	20	11	1	10	16
Rest (lying or sitting)	15	9	14	11	30	1	9	27	18
Mild to moderate activity	50	46	49	67	50	58	55	49	51
Unusual or severe exertion	15	9	14	2	—	11	9	2	6
Mental stres	15	—	1	—	—	—	—	(2)	(1)
Uncertain	15	9	3	—	—	—	9	12	9

21 per cent of the ICS-patients and 18 per cent of the OBS-patients.

Normal physical and mental activities were defined as activities of ordinary daily living such as walking, personal hygiene, household work and ordinary work. About half of the patients in each of the three diagnostic groups reported such activities. There were no statistical d fferences in any of these respects

Heavy physical activity at onset of symptoms was reported by 14 per cent of the AMI patients, 11 per cent of the ICS-patients and 6 per cent of the OBS-patients These differences were not statistic lly significant

One patient in the AMI group developed chest pain when he was taking part n a demonstration Another patient in the OBS-group got her symptoms when iting a pharmacy These two patients themsel es mentioned mental stress at the onset of hest pain.

Three AMI patient and 7 OBS-patients could not adequately def ne their acti ities at onset

Consumption of alcohol during the last 24 hours before adm ssion

Some patients had taken alcohol before the onset of symptoms whereas other had tried alcohol to relieve the pain after onset. The quantity of alcohol was mostly moderate and not estimated in detail.

There was a difference between the sexes in the consumption of alcohol. About a fourth of the men had had an intake of alcohol before admission against about a tenth of the women.

As can be seen in Table 28 the circumstances were about the same in the AMI and in the OBS-group. Two patients in the ICS-group had taken alcohol before admission.

C mment

The findings in this study concerning the ac tiv ties of patients at onset are in accordance to previous studies of (Master 1937 Sievers 1963, Roberts 1972) There was no difference between these patients and the OBS-patients in this respect.

T ABLE 28 Consumpt on kohol dur the last 24 hours before admission the three diagnostic groups

	AMI			ICS			OBS		
	M l	Femal	T tal	Males 9 per ent	Females 1 per ent	Total = 19 pe ent	Males = 43 per ent	Females n = 41 per ent	Total = 81 per ent
I tients ith consumption lcohol					1	1	3	7	16

As previously shown the prevalence of onset of AMI was rather low in immediate connection with physical and mental stress (Master 1937 Billings et al. 1949 Solomon et al 1969) Master and his associates (1937) pointed out that s nce half of the day of normal persons is spent in mild or moderate activity one might well expect half of all attacks to occur during this kind of acti ity and concluded that effort is not a precipitating factor in causing myocardial infarction. Blumgart (1945) did not agree and quoted Bean, who pointed out, the fact that although most motor accidents do not occur *at speeds of 70 miles an hour does not prove* that such speed may not be concerned in some motor accidents. The intake of alcohol within 24 hours before admission was the same in the AMI and OBS-patients and more common in men than in women.

To sum up the activities at onset of symptoms leading the patients to CCU are about the same *in the different groups and gi e no help n the* early diagnosis of AMI

SYMPTOMS AT ONSET

Anterior chest pain is the most prominent symptom in acute myocardial infarction (Heberden, 1786 Herrick, 1912 Bruenn 1936, Bean, 1938 Rosenbaum Levine, 1941 Chambers, 1946 Bidrck 1960 b *Schölmerich, 1962, Reimann and Jahr* märker 1969 Chapman, 1971 Konttinen, 1971 and Simpson and Chetlin, 1971) On the other hand there are innumerabl noncardiac conditions, visceral as well as somatic that give rise to anterio chest pain (Prinzmetal, 1955)

The incidence of *so called painless infarctions* varies from 0 per cent to more than 60 per cent (Babey 1939 Roseman, 1954 Evans and Sutton 1956, Stokes and Dawber 1959 and Lindberg et al. 1960) and there is a tendency in fact that the number of these is decreasing with improved history taking. Better clinical diagnosis will give fewer painless infarctions (Uhlenbruck and Land 1967) It is important to make a distinction between painless and symptomless myocardial infarc tion (Michelmayr and Schweitzer 1967) Rei mann and Jahrmärker (1969) shows in their study that 15 per cent of the AMI patients were w thout pain at onset but only 1 5 per cent without any *symptoms such as dyspnoea, sweating and disturb*ances in consciousness. The problems of denial of chest pain have been studied during the last years (Olin and Hackett, 1964 Croog et al., 1971) It is pparent that one of the reasons for different incidences of painless infarctions is connected with this aspect. Croog et al (1971) showed that in 345 male cardiac patients 20 per cent denied that they had had a heart attack as soon as 3 weeks after their first myocardial infarction. One year later the results were the same suggesting persistence of the denial

In the present study no judgement of the incidence of painless or symptomless infarctions could be made because of our criteria for admission to the CCU (page 11)

Pain

Table 29 shows the patients in the d fferent diagnostic groups admitted on criteria 2—4 (page 11) Nine patients in this study were admitted without pain—3 AMI patients 1 ICS-patients and 5 *OBS-patients*

All other patients gave a history of chest pain

TABLE 29 Patients in the three diagnostic groups admitted on other criteria than chest pain

Cause of admission	AMI Males = 2	AMI Females = 1	AMI Total = 3	ICS Males = 1	ICS Females	ICS Total = 1	OBS Males = 4	OBS Females = 1	OBS Total = 5
Pulmonary oedema	1	—	1	—	—	—	2	1	3
Shock	—	—	—	—	—	—	—	—	—
Syncope	1	1	2	1	—	1	2	—	2

at onset and these have been asked in detail about the quality localization, duration and intensity of the chest pain and about occurrence of radiation of pain to the arms or extension of the pain to other areas than the anterior chest wall No study of anxiety at onset of chest pain was performed.

Types of chest pain

Thirtyfour per cent of the AMI patients used the expression "pain" or ache in their description. The same expressions were also used by 37 per cent of the ICS-patients and by 29 per cent of the OBS-patients. (Table 30)

A type of pain more like oppression was reported by 16 per cent of the AMI patients and 26 per cent of the ICS-patients wh le 37 per cent of the OBS-patients characterized their type of pain in this way The difference between AMI and OBS-patients was significant ($p<0.01$)

A description of a constricting or vicelike pain was given by 18 per cent of the AMI patients, 11 per cent of the ICS-patients and 7 per cent of the OBS-patients. The difference between the AMI and OBS-patients was almost significant ($p<0.05$)

A burning or smarting pain was indicated by 14 per cent of the AMI patients 5 per cent of the ICS-pat ents and 7 per cent of the OBS-pa tients The difference d d not reach significance.

Repeated bouts of pain relieved spontaneously or by nitroglycerine oc urred in 13 per cent of the AMI patients and 16 per cent of the ICS-patients and in 12 per cent of the OBS-patients.

Two per cent in both the AMI and OBS-group indicated discomfort in the chest as a description of their complaints.

Comments

The classification of the quality of the pain is difficult because of semantic difficulties (Feinstein, 1969 and Boyle 1970) The patient s description is built upon previous experiences, different vocabulary and imagination. Several authors have tried to analyze the types of pain in AMI (Bean, 1938 Prinzmetal and Massumi 1955 Keele, 1968 and Sampson and Cheitlin, 1971) The descriptions vary greatly with the countries since the words used are often not easily translatable.

In this study it is shown that chest pain of contricting or vicelike character is somewhat more common in AMI patients while pain described as a pressure is more common in the OBS-patients. The discriminative power of these differences is small since the semantic problems are complicated in this field of the patient-doctor relationship. This is also the reason why no attempt has been made to evaluate the occurrence of anxiety at onset of chest pain. Plügge (1955) has tried to clarify the distinction between chest pain and other types of pains e.g. renal colic. The latter is located peripherally to an organ and can be objectively looked upon by the patient while chest pain interpreted

TABLE 30 Types of chest pai in the three diagnostic groups

	AMI			ICS			OBS		
	Males 66 per cent	Females = 2 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Painles	3	5	5	11	—	5	9	2	6
Pain, ache	33	36	34	33	40	37	26	32	29
P essure	1	3	16	2	30	6	33	41	37
Constriction	15		18	2	—	11	7	7	7*
Burn	5	9	14	—	10	5	12	2	7
Status anginosus		—	13	11	20	16	14	10	12
Discomfort	3			—	—	—	—	5	

p < 0 05 p < 0 01

as cardiac pain is more central more life threatening and existential in its character If this difference of experience is biological or a product of empirical knowledge about the connection between cardiac pain and death is a question of philosophy according to Plügge.

Böörck (1967) considered that the anxiety can be a part of the experience of chest pain, but also an independent psychological reaction associated with the conception that this symptom is an ominous danger signal.

Localization of the pain over the chest

The patients were asked to demonstrate the area of pain over the chest by tracing it with their fingers. The result was registered with regard to site: retrosternally parasternally left or right, retroparasternally or pararetroparasternally (Sïwe 1971)

Pain mainly retrosternally was indicated by 25 per cent of the AMI-patients, 32 per cent of the ICS-patients while 12 per cent of the OBS-patients gave this information. This difference was almost significant ($p<0.05$) (Table 31) Concerning the other sites there were no differences between the three groups.

Only one per cent of the AMI and of the OBS-patients had pain only to the right of the sternum. Pain localized from the left to the right medioclavicular line over the chest was indicated by 45 per cent of the AMI patients, 32 per cent of the ICS-patients and 40 per cent of the OBS-patients.

Extension of chest pain to other areas

Pain extending to the neck was found in 14 per cent of the AMI patients, in 32 per cent of the ICS-patients and in 13 per cent of the OBS-patients (Table 32)

Ten per cent of the AMI patients had pain extending to the jaw while none of the other patients mentioned this extension ($p<0.01$)

Three per cent of the AMI-patients, 16 per cent of the ICS-patients and 5 per cent of the OBS-patients felt an extension of the chest pain to the shoulders

In 15 per cent of the AMI patients there was a less well defined pain in the back in combination with the chest pain. This was also found in 5 per cent of the ICS-patients and in 18 per cent of the OBS-patients.

Pain in the back was more common in women than in men regardless of final diagnosis. Ten per cent of the men had pain in their back against 23 per cent of the women ($p<0.05$)

Some of the patients indicated extension of the pain to several areas.

Radiation of pain to the arms

Radiation of pain to the arms is a referred pain and implies that the patient has an experience of pain in an area separated from the organ which

TABLE 31 Localization of the pain over the chest in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Painless	3	5	3	11	—	5	9	2	6
Retrosternally	26	23	25	11	50	32	9	15	12
Parasternally left	15	9	14	11	—	5	26	20	23
Retroparasternally	8	23	11	33	20	26	14	22	18
Parasternally right	2	—	1	—	—	—	—	2	1
Pararetroparasternally	47	41	45	33	30	32	42	39	40

$p<0.05$

while 19 per cent of the OBS-patients indicated this duration of pain. This difference was significant ($p < 0.01$) (Table 34)

More than half of the AMI patients and half of the ICS-patients had a duration of pain between one hour and six hours while a third of the OBS-patients indicated this duration. This difference was almost significant ($p < 0.05$)

A duration of pain more than six hours did not significantly separate the groups. It was indicated by 41 per cent of the AMI-patients, 39 per cent of the ICS-patients and 46 per cent of the OBS-patients.

Morphine given on admission

Morphine was given to 67 per cent of the AMI patients and 47 per cent of the ICS-patients while 37 per cent of the OBS-patients was given this on admission. The difference between the AMI and OBS-patients was highly significant ($p < 0.001$) (Table 34)

Comments

Yater et al. (1948) have shown that only 8 per cent of the AMI patients in their material had a duration of pain less than one hour. In that study 30 per cent of the AMI patients marked a duration of pain between one and four hours while 53 per cent indicated pain from 4 to 48 hours.

The conclusion of the present study was that a duration of pain less than one hour but more than 15 minutes (se admission criteria page 11) speaks against the AMI-diagnosis while a duration between one and six hours speaks for this diagnosis and a duration of more than six hours was less specific.

The intensity of pain is difficult to estimate. In this study the AMI patients and the ICS-patients were given morphine more often on admission than the OBS-patients. Meltzer (1968) said that 62 per cent of the AMI patients had moderate to severe pain during the attack. The quantification of pain is however very difficult since the pain threshold is different in different individuals. Many authors have therefore tried to subdivide the patients in different groups of sensitiveness with different algometric methods (Libman, 1934). Keele in his report (1968) considered that 23 per cent of the patients were hypersensitive according to his definition. Sixtytwo per cent were normosensitive and 15 per cent hyposensitive. He claimed that the pattern of pain was probably a function of the individual threshold and the noxious stimulation.

Dyspnoea

Several authors have pointed out that dyspnoea is next to pain the most salient symptom at onset of an acute myocardial infarction (Bean, 1938, Rosenbaum-Levine 1941 Billings et al., 1949 and Schölmerich, 1962) furthermore, in some patients

TABLE 34 Duration of pain in the patients with this symptom before admission, and the administration of morphine on admission in the three diagnostic groups

	AMI			ICS			OBS		
	Males 64	Females = 21	Total = 85	Males = 8	Females = 10	Total n = 18	Males n = 39	Females = 40	Total = 79
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Less than 1 h	8	—	6	—	10	6	18	20	19*
1—6 hrs		6	53	63	50	56	33	38	35
More than 6 hrs		33	41	3	40	39	49	4	46
Morphine given on admission		3		44	50	4	33	39	37

$p < 0.05$ $p < 0.01$ p

TABLE 35 The incidence of dyspnoea before adm[illegible] [illegible] diagnostic groups

	AMI			ICS			OBS		
	Males n = 66 per cent	Females = per cent	Total = per cent	[illegible] = [illegible]	[illegible] = per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
No dyspnoea	61	54	5[illegible]		6[illegible]	63	49	46	46
Dyspnoea	36	32	3[illegible]			3[illegible]	44	44	44
Rattling respiration	5	14			—	—	9	10	10

with AMI dyspnoea can be the only sym[illegible] (Master et al., 1937 Roseman, 1954) Bjerk[illegible] 1957 on the other hand, maintains that subject[illegible] impressions of dyspnoea during the acute attack of pain cannot be judged because patients so often confuse dyspnoea with the chest pain of the feeling of oppression that accompanies the pain

In the present study dyspnoea is defined [illegible] symptom indicated by the patient and not [illegible] sign registered by the observer. The dyspnoea was characterized by the patients as shortness of breath or as if they were out of breath. A few patients experienced rattling respiration.

As can be seen in Table 35 35 per cent of the AMI patients had experienced dyspnoea before ad mission and a further 7 per cent rattling respiration Dyspnoea was indicated by 37 per cent of the ICS-pa tients but none of them indicated rattling re piration In the OBS-group 44 per cent had felt difficulties in breathing and 10 per cent felt rattling respiration.

The occurrence of previous heart failure and history of smoking in the patients with or without dyspnoea are shown in Table 36, where also objective signs such as tachypnoea and rales at ad mission are presented.

A history of heart failure was obtained more often in the dyspnoeic groups with a very high incidence in the group with rattling respiration. Previous heart failure was more common in the OBS-group with dyspnoea than in the correspond ing AMI group ($p<0.05$)

As has been shown the patients in the AMI group smoked more than the patients in the other

TABLE 36. The symptom of dyspnoea compared with some objective parameters in the history and the physical examination of the 191 patients in the three diagnostic groups

	AMI			ICS			OBS		
	No dyspnoea = 51 per cent	Dyspnoea = 31 per cent	Rattling respiration = 6 per cent	No dyspnoea = 12 per cent	Dyspnoea = 7 per cent	Rattling respiration = 0 per cent	No dyspnoea = 39 per cent	Dyspnoea = 37 per cent	Rattling respiration = 8 per cent
Previous heart failure	26	39*	100	67	71	—	39	65	[illegible]
Smoker	51	65	33	25	14	—	15	32	38
Respiratory rate >20	[illegible]	51	33	8	[illegible]	—	23	27	87
More than few rales at the lungs	28	48**	83	25	14	—	13	16	100

*$p < 0.05$ **$p < 0.01$

groups (page 38) There were, however no statistical differences between smokers and nonsmokers concerning dyspnoea at onset of symptoms.

Tachypnoea on admission, here arbitrarily defined as a respiratory rate of >20/min. occurred in 54 per cent of the AMI-patients complaining of dyspnoea against 27 per cent of the corresponding OBS-patients, and 29 per cent of the ICS-patients with dyspnoea. The difference between the AMI and OBS-patients in this respect was almost significant ($p<0.05$)

A presence of more than a few scattered basal rales on admission was noted in 48 per cent of the AMI patients with dyspnoea against 16 per cent of the corresponding OBS-patients and 14 per cent of the ICS-patients. The difference between the AMI and the OBS- patients was significant ($p<0.01$)

Nearly all of the patients with rattling respiration had a history of previous heart failure and had rales on the lungs on admission. Two of the AMI patients with rattling respiration had a respiratory rate of more than 20/min. against 7 of the corresponding OBS-patients.

Comments

In the present study dyspnoea is defined as a symptom. This symptom occurred in about the same frequency in the three diagnostic groups. This corresponds to the findings presented in part I (page 17) The subjective difficulty in breathing is often associated with a feeling of constriction or being out of breath. In some patients the dyspnoea is the first and sometimes the only symptom. In other patients the difficulty in breathing appears later on. Libman (1934) postulated that severe pain may cover dyspnoea and on the other hand, marked dyspnoea may cover a moderate amount of pain

The similar frequency of dyspnoea in the three diagnostic groups speak [illegible] multifactorial connection between dyspnoea and other symptoms leading a patient to the hospital

There were however some objective differences between the three groups of patients. Both tachypnoea and occurrence of [illegible] on admission were more common in the AMI patients indicating dyspnoea than in the corresponding OBS-patients.

In the patients of the OBS-group with dyspnoea a history of previous heart failure was more common than in the corresponding AMI-patients.

There were no statistical differences between smokers and nonsmokers concerning dyspnoea at onset.

There are several explanations for this symptom in these patients (Nisell 1967 Rapaport, 1971) One is acute left heart failure secondary to the heart damage (Bean, 1938) But even Christian (1925) pointed out the disproportion between the degree of dyspnoea and any abnormalities demonstrable in the heart and lungs.

Dyspnoea can be a symptom of cardiac neurosis (Björck 1957) with hyperventilation in combination with severe pain and anxiety (Mola, 1971) A third explanation can be the occurrence of acute left heart failure without acute necrosis of any part of the heart.

The different figures presented in the literature may depend on different definitions. Some authors define dyspnoea as a symptom and a sign at the same time while other authors mean only a symptom or a sign Bjerkelund (1957) only considered dyspnoea in cases which on examination had objective signs of dyspnoea or in cases without simultaneous pain.

Bean (1938) showed a incidence of 95 per cent with dyspnoea. In his study more than 25 per cent of the patients had Cheyne Stokes resperation. More than 70 per cent of the AMI patients were reported having dyspnoea by Rosenbaum-Levine (1941) Chambers (1946) and Billings et al (1949) In a Swedish study recently published (Henning and Holmberg 1971) the frequency of dyspnoea and rattling respiration were similar to the findings in the present study concerning the AMI patients.

To sum up dyspnoea is not an useful differential diagnostic parameter for purpose of early evaluation of patients with onset of central chest pain. Some objective signs such as occurrence of basal [illegible]les or high respiratory rate in connection with

dyspnoea can, however increase the validity of this symptom as connected with acute heart damage.

Disturbances in consciousness

The interpretation of symptoms varies with the clarity of perception in each individual (Bean 1938) Sudden clouding of the sensorium or unconsciousness can in rare cases be the only symptom of AMI (Chilazi 1967 Erikssen 1970 Bostrom and Ström, 1971 Carré 1971)

A distinction between clouding of the sensorium or fainting and syncope or unconsciousness was made. As can be seen in Table 37 9 per cent of the AMI-patients had experienced some clouding of the sensorium and 6 per cent had been unconscious. In the ICS-group 37 per cent of the patients indicated disturbances in consciousness and 5 per cent (1 patient) unconsciousness while 19 per cent in the OBS-group had a history of fainting prior to the admission to CCU and 11 per cent had been unconscious. The difference between the AMI and ICS-patients was significant ($p<0.01$) and between the AMI and OBS-patients almost significant ($p<0.05$) If the AMI and ICS-patients together were compared to the OBS-patients there was no statistical difference

Comments

In this study the incidence of fainting and unconsciousness before admission to the CCU was more pronounced in the ICS- and OBS-group than in the AMI group Patients with cerebral lesions, alcoholism diabetic coma or certain active infections may sometimes not adequately register the symptoms of an AMI because of a low perception (Bean 1938) This is also the fact in psychotic patients (Wolpert et al. 1971)

In the present study only patients with a known onset of symptoms within 48 hours are included. The description of the disturbances of consciousness vary but most of the patients said that everything went black or grew dim. Some patients felt a lightheadness The periods of unconsciousness were usually reported as being of rather short duration

The similar incidence of fainting or unconsciousness in the three diagnostic groups indicates grounds to suspect different causes in these patients

Severe pain, emotional stress and vasovagals reactions can be the cause of fainting and syncope at onset of the symptoms leading to hospitalisation (Rushmer 1955 Shillingford 1970) Disturbances of heart rhythm can probably explain some of the episodes of a feeling of fainting

A decrease in heart rate and cardiac output with an associated reduction in peripheral resistance and blood pressure can be the cause of syncope in the AMI-patients due to the acute heart damage. The similarity to the OBS-patients in this respect may however, motivate a suspicion that autonomic mechanisms are activated at the onset of chest

TABLE 37 The incidence of disturbances of consciousness in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Intact sensorium	86	82	85	44	70	58	72	68	70
Clouding of sensorium	9	9	9	44	30	37	19	20	19
Unconsciousness	5	9	6	11	—	5	9	12	11

The difference between the AMI and ICS-patients is significant ($p<0.01$) and between the AMI- and OBS-patients almost significant ($p<0.05$)

TABLE 38 Palpitations correlated to heartrate, blood pressure, pulse pressure and occurrence of atrial fibrillation (AF) on admission and of previous treatment of digitalis in the three diagnostic groups.

		Heart rate		*Blood pressure*		*Pulse pressure*		*Rythm*	*Digitalis*	
				Syst	Diast					
		<60/ min	>100/ min	>160/ mm Hg	>95/ mm Hg	<40/ mm Hg	>70/ mm Hg	AF	No	Yes
AMI										
No palpitation	n = 73	10	22	33	39	11	16	7	50	23
Palpitation	n = 15	—	6	5	8	3	3	2**	6*	9*
ICS										
No palpitation	= 12	—	3	8	5	2	6	1	5	7*
Palpitation	= 7	—	1	4	2	1	3	1	1	6
OBS										
No palpitation	= 44	2	13	25	12	1	16	5	19	25
Palpitation	= 40	2	19	21	12	3	15	13**	20	20

** p.<0.01

pain and anxiety and that this is the most common cause of fainting and syncope in most of the patients.

This symptom therefore gives no diagnostic aid in the early diagnosis of the patients admitted to the CCU as stated in part I. The disturbances of ...s are reported in different series of ...l patients from 3 to 28 per cent (Chambers 1946, Bean 1938) with several authors indicating about 10 per cent (Billings et al., 1949 Reimann 1969 Verdun de Cantogno 1971) Henning and Holmberg (1971) give an incidence of 26 per cent in their study from Gothenburg, Sweden.

Arrhythmic sensations

Arrhythmic sensations were more common in the OBS-patients described in part I (page 18) as compared to the AMI patients (p<0.001) There was also a difference between the sexes in this respect regardless of final diagnosis (p<0.001)

TABLE 39 Arrhythmic sensation before admission reported by the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males n = 9 per cent	Females = 19 per cent	Total = 10 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Rapid regular	5	9	6	22	10	16	7	15	11
Rapid irregular		18	6	11	—	5	23	1	18
Extra beats	5	9	6	11	10	11	12	22	17
Slow regular or irregular	—	—	—	—	10	5	2	2	2
Arrhythmic sensation of any kind	1	36	18	44	50	37	41	51	48

p <0.01 p <0.001

In this part of the study an attempt has been made to correlate these subjective sensations to some objective parameters (Table 38)

The arrhythmic sensations were described as a feeling of a rapid regular or irregular rhythm, slow regular or irregular rhythm or extra beats

Eighteen per cent of the AMI-patients ga e a history of this kind following the onset of symptoms (Table 39) In the ICS-group the incidence of arrhythmic sensations was 37 per cent and in the OBS-group 48 per cent. The difference between the AMI and OBS-group was highly significant ($p<0.001$) as was the difference n this respect between the sexes regardless of final diagnosis ($p<0.001$) Even the d fference between sexes within the AMI-group was significant ($p<0.01$) The differences between the AMI and ICS-patients and between the ICS- and OBS-patients were not significant.

As can be seen in Table 38 only two of the 9 AMI-patients with atrial fibrillation on admission had felt any arrhythmic sensations against 13 of the 16 OBS-patients with this electrocardiographically verified arrhythmia on admission One of the two ICS-patients with atrial fibrillation on admission had experienced palpitations. The difference between the AMI and OBS-patients was significant in this respect ($p<0.01$)

Patients with any type of tachycardia (>100/min) had arrhythmic sensations somewhat more often regardless of final diagnosis ($p<0.05$)

Patients with previous treatment with digitalis had higher incidence of palpitation in the AMI and ICS-groups ($p<0.01$) but not the OBS-group.

No correlation was found between palpitation and a systolic blood pressure above 160 mmHg, a diastolic above 95 mmHg or a pulse pressure above 70 mmHg

Comments

As already shown in part I (page 18) the difference in arrhythmic sensations was highly significant between AMI and OBS-patients. The patients in the ICS-group seemed to have incidence of palpitation which falls between the two other groups The difference in the experience of palpitat on between men and women is highly significan

It s generally accepted that the main reason fo the mortality reduction in the CCUs is improv d diagnosis and treatment of cardiac arrhythmias (Lown et al. 1967 Killip 1968 Meltzer 1968 Sloman 1968 Pantridge and Adgey 1969 Hofvendahl 19 1) and it is probable that in the great maj ry of the very early deaths in AMI, ar hythmias is the responsible mechanism (Wikland 9 For these reasons perhaps more intensive at tion ought to be given to the symptoms of arrhythmic sensations

A has been shown in the present study the AMI patients subjective registration of the action of he heart at the attack corresponds badly to the knowledge of a high incidence of arrhythmias during the initial stage of AMI. This was pointed out already by Bean (1938) but no certain explanation of this has been offered. The palpitation threshold suggested to be high at flegmatic mentality thick chest wall and high age and low at anxiety catechol release and youth (Harrison-Reeves 1968) None of these findings is suggested to be of importance n explanation of the difference between patients with or without AMI. The occurrence of a h gh systolic or diastolic pressure was about the same in the different groups and gave no clues. A pulse pressure above 70 mmHg was associated with about the same incidence of arrhythmic sensations as one below 70 mmHg

In the present series the AMI and ICS-patients on digitalis at time of onset of symptoms more often felt arrhythmic sensations than the corresponding patients without. In contrast no such difference was found in the OBS-patients.

A plausible reason for the discrepancy concerning arrhythmic sensations in patients with and those without AMI could be the more severe pain in the AMI-patients which are pt to minimize the importance of less obvious symptoms.

Any certain explanation for the difference in experience of arrhythmic sensations cannot be given and further investigations in this field ought to be done.

Billings et al. (1949) found arrhythmic sensations in 7 per cent and Henning and Holmberg (1971) gave a higher figure of 26 per cent. Several authors have pointed to a high incidence of palpitation in patients with cardiac neurosis (Björck 1957, Mosa 1971, Furberg 1971) ▼ Atzenhofer (1971) has noticed that even patients with cervical spondylosis may feel arrhythmic sensations more readily probably because of facilitation in the central nervous system and Mann (1970) has pointed out that disturbances of cardiac rhythm and digestion appear together.

To sum up, the noteworthy discovery of a higher incidence of arrhythmic sensations in patients admitted to the CCU without subsequently verification of the AMI-diagnosis as compared to those with a final AMI-diagnosis reported in the series presented in part I (page 18) was confirmed in this prospective study.

Autonomic Symptoms

The patients admitted to the CCU often indicated autonomic symptoms. As has been shown in part I (page 19) there are reasons for separating nausea, or feeling sick, from vomiting and/or sweating. Since one of the causes for the autonomic symptoms especially that of feeling a desire to vomit, nausea, could be intoxication of digitalis (Lely and van Enter 1972) an analysis of the patients has been made with this in mind as can be seen in Table 40. Morphine ought to be of a less importance since the patients in Stockholm with symptoms suggestive of an AMI commonly are referred to hospital by an ordinary ambulance at once without waiting to see a physician.

Nausea

The Swedish word "illamående" corresponds roughly to the latin word nausea and stands for feeling sick or feeling a desire to vomit. This vague symptom occurred as only autonomic symptom in 5 per cent of the AMI patients, 11 per cent of the ICS-patients and 13 per cent of the OBS-patients. The difference between the AMI and OBS-patients was almost significant ($p < 0.05$). Fourteen of the 17 patients indicating this symptom were on digitalis therapy.

Nausea and sweating

Nausea with sweating occurred in 15 per cent of the AMI-patients and 20 per cent of the OBS-patients while none of the patients in the ICS-

TABLE 40 Autonomic symptoms at onset correlated to previous treatment of digitalis in the three diagnostic groups

	n	Nausea	Nausea + Sweating	Vomiting	Vomiting + Sweating	Sweating
		per cent	per cent	per cent	per cent	per cent
AMI						
Total	85	5	15	5	18	30
no digitalis	56		16		1	32
digitalis	32	9	13	—	15	25
ICS						
Total	19	11	—	5	16	26
no digitalis	6	—	—	–	—	30
digitalis	13	15	—	8	23	15
OBS						
Total	[illegible]	13	20	8	11	19
no digitalis	[illegible]	5	1	5	5	31
digitalis		0	20	13	16	9

The difference between patients with nausea with and without digitalis is significant ($p < 0.01$)

group reported this constellation of symptoms. There was no difference between patients with or without digitalis in this respect.

Vomiting

Vomiting with or without a previous feeling of a desire to vomit and without sweating was indicated by 5 per cent of the AMI patients, 5 per cent of the ICS-patients and 8 per cent of the OBS-patients. None of these AMI patients had digitalis while 6 of the 7 OBS-patients were on this therapy. There were no statistical differences.

Vomiting and sweating

Vomiting and sweating in combination occurred in 18 per cent of the AMI-patients, in 16 per cent of the ICS-patients and in 11 per cent of the OBS-patients. There was no statistical difference between undigitalized and digitalized AMI and OBS-patients.

Sweating

Sweating as an isolated autonomic symptom was reported by 30 per cent of the AMI patients and 26 per cent of the ICS-patients while 19 per cent of the OBS-group indicated this symptom. Sweating alone was more common in undigitalized OBS-patients than in OBS-patients with digitalis ($p < 0.05$) while there were no such differences in the other two diagnostic groups.

Combinations of autonomic symptoms

Nausea with sweating, vomiting with or without sweating and sweating alone are the constellations of symptom called combination of autonomic symptoms in part I (page 19). This was indicated by 68 per cent of the AMI patients in the present material against 47 per cent of the ICS-patients and 58 per cent of the OBS-patients. The difference between AMI and OBS-patients was not significant. Combinations of autonomic symptoms was a little more common in the undigitalized patients in the AMI-group than in the patients with digitalis ($p < 0.05$). If the constellation of nausea and sweating was excluded there was an almost significant difference between the AMI and OBS-patients ($p < 0.05$).

Comments

Autonomic symptoms in the form of nausea, vomiting and sweating are common in patients admitted to CCU (part I, page 19). In the present study the different autonomic symptoms have been studied separately.

Patients indicating vomiting have mostly felt nausea before and/or after the vomiting. As demonstrated in part I nausea or feeling sick as an isolated symptom was more common in the OBS-patients than in the AMI patients. Most of the patients with this single symptom were on digitalis.

Vomiting, sweating and combination of these two symptoms occurred more often in the AMI patients than in the OBS-patients. With the exception of slightly higher prevalence of sweating in undigitalized OBS-patients than in OBS-patients with digitalis there were no differences between patients with or without digitalis.

There are several conceivable explanations of autonomic symptoms in the patients admitted to CCU. Digitalis intoxication can be one (Lely and van Enter, 1972) whereas morphine ought to be of a less importance since only a few of the patients in the present study had seen a physician before admission to the hospital.

Nausea, or feeling sick is a symptom of a more psychological nature according to Wang and Borison (1950, 1953) while vomiting is a more complicated symptom (Harrison et al. 1965). Vomiting stimuli fall into two groups: those acting primarily by enhancing the activity of the vomiting center ("central" vomiting) and those arising in the peripheral part of the body and conveyed to the vomiting center ("reflex" vomiting). Both nausea and vomiting can be due to heart failure and the hepatic and gastrointestinal congestion secondary to failure. Hatcher and Weiss (1927) proposed the theory that autonomic symptoms in acute myocardial infarction were due to a strong medullary bombardement of impulses from the heart.

The incidence of autonomic symptoms at the early stage of AMI is generally rather high in different series. Bean (1938) recorded an incidence

pressure was highly significant lower in the AMI group, thus giving a discriminating factor in the early judgement of the patients on admission to the CCU

Heart rate on admission

Table 42 shows the heart rate and rhythm in the patients on admission. A sinus rhythm below 60/min. was noted in 11 per cent of the AMI patients and in 6 per cent of the OBS-patients while none of the ICS-patients had this heart rate. A sinus rhythm above 100/min. was registered in 22 per cent of the AMI patients and 19 per cent of the OBS-patients against 11 per cent of the ICS-patients.

One AMI patient and 2 OBS-patients had atrial flutter with a heart rate above 140. Three OBS-patients had a permanent pacemaker

Atrial fibrillation on admission was noted in 9 AMI patients, 2 ICS-patients and 16 OBS-patients.

Comments

The heart rate and rhythm on admission were of no s d in differentiating the patients in the three diagnostic groups.

TABLE 42 Heart rate and rhythm on admission in the three diagnostic groups

	AMI	ICS	OBS
	n = 85	n = 19	n = 81
	per cent	per cent	per cent
Sinus rhythm			
< 50	3	—	1
50— 59	8	—	5
60— 79	20	3	21
80— 99	35	4	30
100—119	15	11	15
120—139	5		2
>139			2
Pacemaker			4
Atrial fibrillation			
<100			1
100—119			
120—139			6
>140			1
Atrial flutter			
>140			

TABLE 43 Respiratory rate on admission in the three diagnostic groups

		AMI	ICS	OBS
		n = 88	n = 19	n = 81
		per cent	per cent	per cent
Respiratory rate	<16	5	5	21
	16	19	21	18
	18	10	5	12
	20	30	53	18
	22—21	21	11	13
	≥26	16	5	18

** $p < 0.001$

Respiratory rate on admission

In the present study only 5 per cent of the AMI patients and 5 per cent of the ICS-patients had a respiratory rate below 16/min against 21 per cent of the OBS-patients. The difference between the AMI patients and the OBS-patients was highly significant ($p < 0.001$). An increased respiratory rate above 20/min was noted in 37 per cent of the AMI patients, in 16 per cent of the ICS-patients and in 31 per cent of the OBS-patients. There was no statistical difference in this respect between the AMI and OBS-patients (Table 43)

Pulmonary rales on admission

In more than a third of the AMI patients and a third of the ICS-patients there were more than a few scattered basal rales at the lungs on admission, while this sign was found in a fourth of the OBS-patients ($p < 0.05$) (Table 44)

Comments

A low respiratory rate <16/min. on admission speaks against the AMI-diagnosis but a respiratory rate above 20/min. does not discriminate between the three diagnostic groups. In the AMI and ICS-groups basal rales occur more often than in the OBS-group suggesting a higher left ventricular filling pressure in the former two groups

TABLE 44 The prevalence of pulmonary rales or oedema on admission in the three diagnostic

	AMI = 88 per cent	ICS = 19 per cent	OBS = 84 per cent
No or few basal rales	61	63	77
More than few basal rales	36	37	17
Frank pulmonary oedema	2	—	6

$p<0.05$

Sensorium on admission

Two AMI-patients were unconscious on admission and 4 were mentally confused. Two ICS-patients and 4 OBS-patients were also mentally confused on admission. There were no differences between the groups.

The interviews of these patients were done later when they had recovered their mental faculties or with the assistance of a relative.

E. TESTS ON ADMISSION

Enzyme determinations on admission

A detailed analysis of the enzyme determinations in the patients in the present series is presented in part III (page 26). The normal range for the S-GOT values is 10—35 U/l in this hospital and 10—50 U/l for CPK (Bergström and Sawe, 1972).

About half of the AMI patients had a S-GOT value above 30 U/l on admission while none in the ICS-group had S-GOT value above this limit. Fifteen per cent of the OBS-patients had also an elevation above 30 U/l of the S-GOT on admission. The difference between the AMI patients and the other two groups was highly significant ($p<0.001$).

A CPK value above 50 U/l on admission was found in 44 per cent of the AMI-patients but also in fourth of both the ICS- and OBS-patients. The difference between the AMI and the OBS-patients was highly significant ($p<0.001$) and between the AMI and the ICS-patients significant ($p<0.01$).

Comments

An elevated value of S-GOT or CPK on admission supports the AMI-diagnosis. About half of the AMI patients had a slight elevation of the enzymes on admission but even some of the OBS-patients had this. The conclusion must be that an elevated S-GOT or CPK value on admission does not necessarily lead to a subsequent AMI diagnosis nor does a normal value on admission exclude the diagnosis. The enzyme value in an AMI depends on the time between onset of symptoms and the sampling. There is a certain loss of time in the laboratory procedures meaning that the enzymatic test is not especially valuable as a good discriminative factor in the Casualty Department.

ECG on admission

A third of the AMI-patients had signs suggestive of an AMI according to the Minnesota code on the admission ECG. None of the patients in the other groups showed this, ($p<0.001$) but nearly all of the ICS-patients and 75 per cent of the OBS-patients had ECG with signs of previous infarction, branch bundle block or non-characteristic ST T-changes. Only a fourth of the OBS-patients and a tenth of the ICS-patients had normal admission ECG while none of the AMI patients had this on admission. The difference between the AMI patients and the OBS-patients was highly significant ($p<0.001$) and between AMI and ICS-patients almost significant ($p<0.05$).

C mment

An admission ECG suggestive of an AMI is of course a useful aid in the early diagnosis of AMI. Many of the patients admitted to the CCU have pathologically changed ECG on admission and this may complicate the early evaluation of the ECG. Short (1970) has shown that in the early stages of slight or subacute myocardial infarction, classical pattern such as major ST elevation, abnormal Q waves and obvious ST and T wa e changes are seen in only a minority of cases, and he stated that the cardiogram must be interpreted together with the full clinical picture.

SUMMARY

An analysis of 191 patients was performed in order to try to improve the *early* diagnostic accuracy in patients admitted to a CCU

A detailed questionnaire was constructed to cover systematically prodromal symptoms and acute symptoms and events leading to hospitalisation. Furthermore, the history of previous diseases was included as were some simple clinical parameters on admission.

The incidence of previous angina pectoris and myocardial infarction was about the same in the AMI and OBS-groups while it was considerably higher in the ICS-group. Previous hypertension was as common in all three groups of patient. The occurrence of diabetes mellitus was high in the ICS-group compared to the other two groups. Peptic ulcer renal calculus and gall stone were common previous conditions in the patients regardless of the final diagnosis.

The incidence of a positive history of smoking was higher in the AMI-group than in the two other groups. This was the case in spite of a high incidence of IHD-manifestations even in these two latter groups.

New or increasing chest pain, unstable angina, was especially discriminating between the AMI and the OBS-group

In the AMI group there was a slightly higher onset of the symptoms during the weekends than in the two other groups. A delay of less than 6 hours was more common in the AMI and ICS-groups than in the OBS-group The activities at onset of symptoms were about the same in the different groups.

In the present study an analysis of different parameters of the chest pain has been made The problems concerning the occurrence of painless and symptomless infarction have been discussed.

Constricting or vice-like pain is more common in the AMI patients while pain more like an oppression more often occurred in the OBS-patients The localization of the pain over the chest was about the same in all three groups of patients. Ten per cent of the AMI patients indicated extension of the pain to the jaw while none of the other patients mentioned this. Radiation of the pain to the arms was much more common in the AMI and the ICS-patients than in the OBS-patients. A short duration of pain, less than one hour was more often indicated by the OBS-patients than by the other two groups of patients.

Dyspnoe or disturbances of consciousness were not useful differential diagnostic parameters for purpose of early evaluation of patients with onset of central chest pain since it occurred with about the same frequency in all three diagnostic groups.

The noteworthy discovery of a higher incidence of arrhytmic sensations in patients admitted to the CCU without subsequent verification of the AMI diagnosis as compared to those with a final AMI diagnosis reported in the series presented in part I was confirmed in this prospective study

The incidence of autonomic symptoms is high in patients admitted to the CCU Nausea, or feeling sick as an isolated symptom occurred more often in the OBS-patients especially in those with digitalis, while vomiting and/or sweating were more common in the AMI patients.

Three patients—all in the AMI group—reported hiccuping

In the present series blood pressure heart and respiratory rate and occurrence of basal rales have been registered on admission and ECG and enzyme-determinations have been performed on admission to the CCU

There were *no significant differences* concerning the systolic or diastolic pressure on admission between the three groups of patients but a pulse pressure below 60 mm Hg was more common in the AMI group than in the two other groups.

The heart rate and rhythm on admission were of no aid in the early differentiation of the patients in the three diagnostic groups.

A low respiratory rate on admission less than 16 per minute was more often registered in the OBS-patients than in the two other groups of patients.

About half of the AMI patients had a slight elevation of the S-GOT value on admission but

even a few of the OBS-patients had this. Since there is a certain loss of time in the laboratory procedures the enzymatic test is not especially valuable as a good early discriminative factor in the Casualty Department.

A third of the AMI patients had signs suggestive of an AMI on the admission ECG and none of the patients in the two other groups.

These findings are the basis of the attempt to work out a diagnostic index presented in part V

Diagnostic Index based on the Early Clinical Picture

The purpose of this section of the present study is to try to construct a better clinical diagnostic index than that described in part II (page 21).

The early clinical picture described in the patients presented in part IV was the basis of this attempt.

MATERIALS AND METHODS

The 191 patients presented in part IV were divided in two groups. One group was made up of the 88 AMI patients and the 19 ICS-patients and another of the 84 OBS-patients.

Nine parameters significantly discriminating the 107 patients in the former group from the 84 patients in the latter group were used in the discriminant function analysis. From the history and the acute symptoms the following parameters were chosen: history of smoking, history of unstable angina pectoris, radiation of pain to the arms, extension of pain to the jaw, a duration of pain shorter than one hour and sensations of arrhythmia.

From the physical examination a pulse pressure below 60 mm Hg and a respiratory rate below 16 per minute were chosen as discriminating factors.

An admission ECG suggestive of an AMI is an important early discriminating factor and this parameter was also included.

Dizziness as a prodrome was judged to vague, as was the type of chest pain at onset and these two parameters were excluded from the analysis.

The enzyme value on admission as a discriminating factor was excluded since there is a certain loss of time in the laboratory procedures.

The same statistical methods as described in part II are used (page 21).

RESULTS

The discriminant function coefficients of the nine parameters are presented in Table 45. For arithmetical ease all coefficients were multiplied by 10^3 and rounded to the nearest whole number.

The score was obtained by addition of the existing parameters in each patients. Fig. 8 shows the histograms of these discriminant scores of the patients in the two groups.

Patients who died during the hospital stay are shaded in Fig. 8 in order to show that this diagnostic index does not represent a prognostic index.

Specificity at different levels of sensitivity was calculated according to the formulae presented in part II (page 21) and graphically illustrated in Fig. 9.

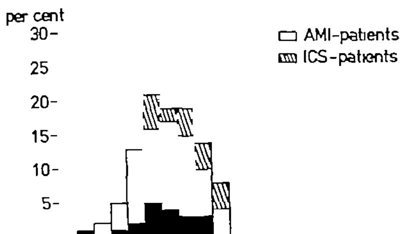
At an index score below −5 the sensitivity was 100 per cent with a specificity of 16 per cent.

TABLE 45. Discriminant function coefficient derived from the discriminant function analysis

+0.001	history of smoking	+ 4
+0.00596	unstable angina	+ 6
+0.00516	radiation to the arm	+ 5
+0.007[illegible]3	extension of pain to the jaw	[illegible]
−0.00509	duration of pain ≤ 1 hour	5
−0.005[illegible]9	arrhythmic sensation	6
+0.00159	pulse pressure < 60 mm Hg	[illegible]
−0.00759	respiratory rate < 16/min	8
+0.01119	admission ECG suggestive of AMI	11

DISCUSSION

As has been pointed out in part II of the present study a discriminant function analysis of the kind performed in the present study ought to be seen [illegible] an attempt to identify [illegible] symptoms and signs which are essential for the description of a disease and more important for differentiation between diseases.



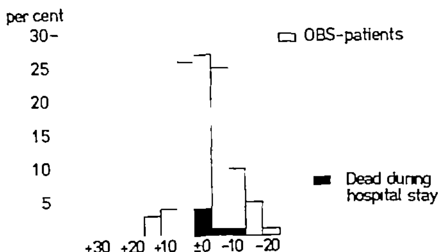


Fig. 8 Distribution of the clinical diagnostic index in the AMI- and ICS-patients compared to that of the OBS-patients The scores are derived from the discriminant function analysis. (Black areas represent patients dead during the hospital stay)

Bruce and Yarnall (1966) stated that in the physician—computer interaction the computer will not relieve the physician of his responsibility of making decisions regarding each specific patient. The effect of this interaction is, according to these authors, the motivation of the physician to attempt to be more rigorou in defining concepts, in col lecting information, and in making reasonable decisions.

"Next there is the fact that what is easy for the computer may be difficult for men, and what is easy for men may be extremely difficult for the computer (Sterling and Pollack, 1965)

Cohn and Gorlin (1972) stressed that this type of indices does not represent a diagnostic panacea, but it does give a diagnostic guideline

In the present study it has been shown that a history of smoking and a history of unstable angina defined according to the Edinburgh group (Fulton et al., 1971) are parameters which increase the probability of an AMI in a patient with an attack of chest pain lasting more than one hour and with radiation of pa n to the arms and/or extension of pain to the jaw A pulse pressure below 60 mm Hg on admission also increases the probability of an AMI If the patient has had

KAMARYT P MINARIK, J & MIKLIS, P Total delay bet een f rst appearance of symptoms in and hospitalization of patients ith acute myocardial infarction *Cor I si 14 1 197*

KEELE, K. D Pain complaint threshold in relation t pain of cardiac inf rction. *Brit Med J 1 670 1968*

KENDALL, M G & STUART A. The advanced theory of statistics. I *ol 1 315 London 1966.*

KIBE, O & NILSSON N J Observations on the diagnostic and prognostic lue of some enzyme tests in myocardial infarction. *Acta Med Scand 182 597 1967*

KILLIP T. Acute myocardial infarction. Eds. D G Julian and M F Ol er *E S L gston Ltd Great Britain 1968*

KONTTINEN A. How to be sure it myocardial infarction *Medical Times 99 12 p 49 1971*

KRAUSS, K. R, HUTTER, J A. M. & DESANCTIS, R. W Acute coronary insufficiency course and follow-up *Circulat 45—46 pN I 66 1972*

KULLER, L Sudden and unexpected non traumatic deaths in adult. review of epidemiological and lincal studies *J Chr ic Di 19 1165 1966*

LELY A. H & VAN ENTER, C. H J Non-cardiac symptom of digitalis intoxication *Amer H art J 83 149 197*

LEMLICH A M h riant analysis of clinical and prognostic factors in myoc rdial inf rction. *N Y York J Med 65 1709 1965*

LEVINE, H J Pre-inf rction syndrome *Amer J Cardiol 2 31 1971*

LIBMAN E Observ tions on indi idual sensitiveness to pain With special referenc to abdominal disorders *JAMA 102 335 1934*

LINDBERG H A BERKSON D M STAMLER, J & POINDEXTER, A Totally symptomatic myocardial inf rction An estimate of its incidenc in the living population *Intr Med 106 628 1960*

LINDHOLM, H On the ration of the time of onset and of death of myoc rdial infarction *Acta Med Scand 1 5 3 1965*

LOWN, B VASSAUX C HOOD W FAKHRO A M KAPLINSKY E & ROBERGE G Unresol ed problem in oronary re *A er J Card ol 20 494 196*

LUNDMAN T MOGENSEN L & ORINIUS E D tamental t e patienter som findas pa hjartinf rkta *Stockholm 1968*

LUNDMAN T MOGENSEN L NORDENSTAM H & ORINIUS E Tet reatheter (...)

LUSTED L B ...

LUSTED L B Decision-making studies in p ... Med ...

MACKENZIE J Symptoms and their interpretation. *5th ed Sev London 1909*

MANN D Zusammenhange zwischen Herzrhythmusstorungen und Vorgangen im Verdauungstrakt *Z G I n M d 6 86 1971*

MASTER, A. M DACK, S. & JAFFE, H L. Coronary thrombosis an investigation of heart failure and other factors in its course and prognosis *Amer H art J 13 330 1937*

MASTER, A. M DACK, S & JAFFE, H L. Factors and events associated ith onset of oronary rtery thrombosis. *JAMA 199 316 1937 b*

MASTER, A. M JAFFE, H L, FIELD L E & DONOSO E. Acut coronary insufficiency Its differential diagnosis and treatment *A I tern Med 45 561 1956.*

MAURICE, P BEAUMONT J L LEUPIN A. & LENEGRE, A. & LENEGRE, J La période prémonitoire de l'inf rctus d myocarde *Société f ançai d Cardiol gie Séanc d 6 octobre 1954*

MELTZER, L. E. Acute myocardial infarction. Ed D G Julian and M F Ol er *E S Livi gstone Ltd Great Britain, 1968*

MICHELMAYER, G & SCHWEITZER, F Der ty p sche Herzinf rkt *W e Med W ochenschr 117 66. 1967*

MILLER, H R Angina pectoris. *W illiams H ... Medical Book Ltd London 194*

MOIA, B Cardiac neuroses Recognition and management *Cardi c Clin 21 1971*

MOSS, A J WYNAR, B & GOLDSTEIN S Delay in hospitalization during the acut oronary period *Amer J Card ol 24 659 1969*

MOSS A J & GOLDSTEIN S The pre-hospital phase of acute myocardial infarction *Circulation 41 37 19 0*

MOU SEY P Prodromal symptoms in myoc dial inf rction *Bri H art J 13 215 1951*

NISELL, O Dyspné *M d rn k A bok 10 79 1967*

NOZAWA T Studies on serum enzymes in cardiovascular diseases with pecial reference to serum lactic dehydrogenase isoenzymes *J p Cir J 34 237 1970*

NYQUIST O Shock omplic ting acute myocardial infarction *Act Med Scand S pl 33 19*

OLIN H S & HACKETT T P The denial of chest pain in 32 pat ent w h acute myoc dial inf rction *JAM 1 190 103 1964*

PANTRIDGE J F & ADGEY A A J Pre-hospital oronary re The mobile oronary re unit *Amer J Card ol 4 666 1969*

PIPBERGER H V KLINGEMAN J D & COSMA, J Computer ev luation of st al properties of ... in the differential diagnosis of ... Med 9 1968

FLOTZ, M. *Coronary heart d as pp 10 —117 Hoeber N w York 1957*

FLÜGGE, H. Über Herzschmerzen — Ein phaenomenologischer Versuch — *Arztl W schr* 10 *133 1955*

POGGI, L., BORY M., DJIANE, P CHABAUD R SEBAOUN, E. & SERRADIMIGNI A Les troubles de rythme au cours des crises d'angor spontané de Prinzmetal. *Arch Mal Coeur* 64 *874 1971*

POPESCU I ZAMFIRESCU-GHEORGHIU M. & GAVRILA, F Contribution to the enzymic diagnosis of acute myocarditis, myocardial infarction and cardiogenic shock. *Cardiol gia* 54 *278 1969*

PRINZMETAL, M & MASSUMI R. A The anterior chest wall syndrome—chest pain resembling pain of cardiac origin. *JAMA* 159 *177 1955*

PRINZMETAL, M., EKMECKI, A TOYOSHIMA, H & KWOCZYNSKI, J K.. Angina Pectoris III Demonstration of chemical origin in ST deviation in classic angina pectoris, its variant form, early form, early myocardial infarction, and some noncardiac conditions. *Amer J Cardiol* 3 *276, 1959*

PUDDU V A case of acute myocardial infarction with an atypical symptomatology Cutaneous itching *Amer H art J* 82 *431 19 1*

RAPAPORT E Dyspnea. Pathophysiology and differential diagnosis. *Prog Cardiovasc Dis* 13 *532, 1971*

REIMANN R. & JAHRMÄRKER, H Zur Klinik des Herzinfarktes. Symptomatologie und Komplikationen *Münch Med Wschr* 111 *923 1969*

RENGGLI, I Prodrome beim akuten Myokardinfarkt *Schweiz Med Wschr* 101 *133 1971*

RESNIK, W H Preinfarction angina Part I the transaminase test— diagnostic aid I *Mod Conc Cardi as Di* 31 *751 1962*

RESNIK, W H Preinfarction angina Part II an interpretation II *Mod Conc Cardio as Di* 31 *757 1962 b*

REUSCH, C. S & BUNCH, L D Heat stable LDH. Heat stable lactic dehydrogenase in blood serum as diagnostic method *J Kans Med Soc* 69 *43 1968*

ROBERTS, W C Coronary arteries in fatal acute myocardial inf rction *Circulation* 45 *214 197*

ROSE, G A The diagnosis of ischaemic heart pain and intermittent claudication in field surveys *Bull. WHO* 27 *645 1962*

ROSEMAN M D Painless myocardial infarction. A review of the literature and analysis of 70 cases. *Ann Intern Med* 41 *1 1954*

ROSENBAUM F F & LEVINE, S. A Prognostic alue of rious linical and electrocardiographic features of acute myocardial infarction *Arch Intern Med* 68 *913 1941*

ROWAN T Enzyme changes in myocardial infarction *W Med J* 68 *777 1969*

RUEGSEGGER, P NYDICK, I FREIMAN A. & LADUE, J S Serum activity patterns of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase following graded myocardial infarction dogs. *Circ Res* *4 1959*

RUSCHMER, R F Diagnosis of cardiac disease. pp 300—305 *Saunder Philadelphia, 1955*

SAMPSON J J & ELIASER, J M Diagnosis of mpending acute coronary artery occlusion. *Amer H art J* 13 *675 1937*

SAMPSON, J J & CHEITLIN, M D Pathophysiology and differential diagnosis of cardiac pain. *Pr g Car d as Di* 13 *507 1971*

SCANLON P J NEMICKAS, R TOBIN J R., ANDERSON, W MONTOYA, A. & PIFARRE, R. Myocardial revascularization during acute phase of myocardial infarction *JAMA* 218. *707 1971*

SCHNEIDER, K. W Klinik und Therapie des Mikroinfarktes. *Wie Klin W sche hr* 118 *143 1968*

SCHÖLMERICH, P Herzschmerz und Herzsensationen. *Monat kurs f ärztl Fortbild* 12. *223 1962*

SCHÖLMERICH, P Klinik des Myokardinfarktes *Deut Med J* 13 *477 1962.*

SHILLINGFORD J P Syncope *Amer J Card ol* 26 *609 1970.*

SHORT D The earliest electrocardiographic evidence of myocardial infarction *Brit Heart J* 32 *6, 1970*

SHUBIN H AFIFI, A. A., RAND W M & WEIL, M H Objective index of hemodynamic status for quantitation of severity and prognosis of shock complicating myocardial infarction. *Cardi Res* 4 *379 1968*

STEVERS, J Myocardial infarction. Clinical features and outcome in three thousand thirty six cases *Acta Med Scand* 5 *ppl 406 1964*

SIMONSON, E Russian research on the role of isceral reflexes in oronary insufficiency *Circulat on* 22 *1179 1960*

SKJAEGGESTAD O Prodromer ed akutt hjerteinfarkt. *Nord Med* 7 *1478 1971*

SLOMAN G Acute myocardial infarction. Eds D G Julian and M. F Oli er *E a S Livingstone Ltd Great Britain 1968*

SOBEL, B E & SHELL, W E. Serum enzyme determinations in the diagnosis and assessment of myocardial infarction. *Circulation* 45 *471 1972.*

SOLOMON, H A., EDWARDS, A. L. & KILLIP T Prodromata in acute myocardial infarction. *Circulation* 40 *463 1969*

STEJFA, J M., MOCEK, J & DVORAK, I On imminent infarction *Cardiol gia* 40 *4 1962.*

STERLING, D & POLLACK, S Computers and the *hf sciences Columbus Univ pres N Y 1965*

STOKES, J & DAWBER, T R. The silent coronary the frequency and clinical characteristics of unrecognized myocardial infarction in the Framingham study *Ann Intern Med* 50 1359 1959

STOWERS, M. & SHORT D Warning symptoms before major myocardial infarction. *Brit H art J* 32 833 1970.

STRÖDER, U BECKER, F & HAAS G. Über die Abhängigkeit des Herzinfarktelntrittes vom Wetter geschehen, T geirythmus und von solaren Vorgängen. *Klin Wochenschr* 17/18 312 1951

SAWE, U Pain in acute myocardial infarction. A study of 137 patients in coronary care unit. *Acta Med Scand* 190 79 1971

TJOE, S L & LURIA, M. H Delays in reaching the cardiac care unit An analysis. *Chest* 61 617 1972.

THOMAS, M., JEWITT D E & SHILLINGFORD J P Analysis of 150 patients with acute myocardial infarction admitted to an intensive care and study unit. *Brit Med J* 1 787 1968

UHLENBRUCK, P & LAND H Der Stumme Herzinfarkt. Bemerkungen zur klinischen Diagnostik des Infarkts. *Landarzt* 43 1138 1967

WAHLBERG, F A study of acute myocardial infarction at the Seraphimer Hospital during 1950—1959 *Amer H art J* 65 749 1963

VAKIL, R J Intermediate coronary syndrome *Circulation* 4 557 1961

WALLACE, A. G KLEIN R F KLINER, V ZIPES, D & ANDREOLI, K. Coronary care units, their goals, current experience and future *Cardiologica* 50 337 196

WANG, S. C & BORISON H. L The emeting center A critical experimental analysis *A h Neurol P ychiat* 63 928 1950

WATKINSON, G The relationship of chronic peptic ulcer t coronary sclerosis (Summary) *Gastroenterology* 85 701 1956

VERDUN DI CANTOGNO L, VIZZERI, E & ORIONE, G Considerazioni sulle modalità di comparsa sulle caratteristiche della crisi infartuale in 500 infarti del miocardio. II Semeiotologia clinica *Minerva Med* 62 1618 1971

VERGHESE, A. & LOVELL, R. R. H. Classification of chest pains after myocardial infarction. *Brit Med J* 11 1102 1966.

WHITE, P D & BLAND E F A further report on the prognosis of angina pectoris and of coronary thrombosis. A study of fiv hundred cases of the former condition and of two hundred cases of the latter *Amer Heart J* 1 1931

W.H O Regional Office for Europe The prodromal symptoms of myocardial infarction od sudden death. *Copenhage* 1971

WIKLAND B Medically unattended fatal cases of ischaemic heart disease in defined population. *Acta Med Scand S ppl* 5 4 1971

WILKINSON J H. The future of diagnostic enzymology *Materia Medica Polona* 3 33 1971

WOLPERT A., YARYURA-TOBIAS, J A. & KERTZNER, L Silent myocardial infarction in chronic psychotic population. *Dis Nerv Syst* 32 780 1971

WYBURN MASON R. Significance of the reference of anginal pain to the right or left side of the body *Amer Heart J* 39 375 1950.

YATER, W M., WELSH P P STAPLETON J F & CLARK, M. L Comparison of clinical and pathologic aspects of coronary artery disease in men of various ge groups study of 950 autopsied cases from the armed forces institute of pathology *A Intern Med* 34. 352 1951

YU P N Prehospital car of acute myocardial infarction *Circulation* 45 189 1972

c

STOKES, J & DAWBER, T R. The silent coronary the frequency and clinical characteristics of unrecognized myocardial infarction in the Framingham study *Ann Intern Med* 50 1359 1959

STOWERS, M. & SHORT D Warning symptoms before major myocardial infarction. *Brit Heart J* 32 833 1970.

STRÖDER, U BECKER, F & HAAS, G. Über die Abhängigkeit des Herzinfarkteintrittes vom Wetter geschehen, Tagesrythmus und on solaren Vorgängen. *Klin Wschensch* 17/18 312, 1951

SÄWE, U Pain in acut myocardial infarction. A study of 137 patients in coronary care unit. *Acta Med Scand* 190 79 1971

TJOE, S. L. & LURIA, M. H. Delays in reaching the cardiac care unit: An analysis. *Chest* 61 617 1972

THOMAS, M, JEWITT D E. & SHILLINGFORD J. P Analysis of 150 patients with acute myocardial infarction admitted to an intensive care and study unit. *Brit Med J* 1. 787 1968

UHLENBRUCK, P & LAND H. Der Stumme Herzinfarkt. Bemerkungen zur klinischen Diagnostik des Infarkts. *Landarzt* 43 1158 1967

WAHLBERG F A study of acute myocardial infarction at the Seraphimer Hospital during 1950—1959 *Amer Heart J* 65 749 1963

VAKIL, R. J Intermediate coronary syndrome. *Circulation* 24. 557 1961

WALLACE, A. G KLEIN, R. F KLINER, V ZIPES, D & ANDREOLI, K. Coronary care units, their goals, current experience and future. *Cardiologia* 50 337 1967

WANG, S. C & BORISON, H. L The vomiting center. A critical experimental analysis. *Arch Neurol. Psychiat* 63 928 1950.

WATKINSON, G The relationship of chronic peptic ulcer t coronary sclerosis (Summary) *Gastroenterol gia* 85 201 1956.

VERDUN DI CANTOGNO L, VIZZERI, E. & ORIONE, G Considerazioni sull modalità di comparsa sulle caratteristiche della crisi infartuale in 500 infarti del miocardio II Sintomatologia clinica. *Minerva Med* 62 1618 1971

VERGHESE, A. & LOVELL, R. R. H Classification of chest pains after myocardial infarction *Brit Med J* 11 1102, 1966

WHITE, P D & BLAND E. F A further report on the prognosis of angina pectoris and of coronary thrombosis. A study of five hundred cases of the former condition and of two hundred cases of the latter. *Amer H art J* 7 1 1931

W.H.O Regional Office for Europe. The prodromal symptoms of myocardial infarction and sudden death. *Copenhagen* 1971

WIKLAND B. Medically unattended fatal cases of ischaemic heart disease in defined population. *Acta Med Scand S ppl* 524 1971

WILKINSON J H. The future of diagnostic enzymology *Materia Medica Polona* 3 35 1971

WOLPERT A., YARYURA TOBIAS, J A. & KERTZNER, L. Silent myocardial infarction in chronic psychotic population. *Dis Nerv Syst* 32 280 1971

WYBURN-MASON, R. Significance of the reference of anginal pain to the right or left side of the body *Amer Heart J* 39 325 1950

YATER, W M, WELSH, P P STAPLETON, J F & CLARK, M. L. Comparison of clinical and pathologic aspects of coronary artery disease in men of various age groups study of 950 autopsied cases from the armed forces institute of pathology *Ann Intern Med* 34 352, 1951

YU P N Prehospital care of acute myocardial infarction. *Circulation* 45 189 1972

cta Medica Scandinavica

lementum 544

lult Human Adipose Tissue
llularity and Metabolism

Special Reference to Obesity and
Acid Synthesis *de Novo*

Lars Sjöström

uted by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

Supplementum 544

Adult Human Adipose Tissue Cellularity and Metabolism

with Special Reference to Obesity and Fatty Acid Synthesis *de Novo*

By Lars Sjöström

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

Subscription Rates

Per annum — two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw kr 170, incl. postage
Other countries Sw kr 200 incl. postage.

Chief Editor

Professor Jan Waldenström MD
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Riddargatan 12 B
S-114 35 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26 Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell
Informationsindustri AB
S-751 81 Uppsala, Sweden

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 544

FROM THE DEPARTMENT OF MEDICINE I, SAHLGREN'S HOSPITAL,
UNIVERSITY OF GÖTEBORG, GÖTEBORG SWEDEN

ADULT HUMAN ADIPOSE TISSUE CELLULARITY AND METABOLISM

with Special Reference to Obesity and Fatty Acid Synthesis *de Novo*

BY

LARS SJÖSTRÖM

GÖTEBORG 1972

This thesis is based on the following papers:

I Sjöström, L Björntorp P & Vrana J : Microscopic fat cell size measurements on frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells J Lipid Res 12:521 1971

II Sjöström L Björntorp P & Månsson J E : An optimal assay system for subcellular determination of de novo fatty acid synthesis in human adipose tissue

III Björntorp P Bengtsson C Blohmé G Jonsson A Sjöström L Tibblin E Tibblin G & Wilhelmsen, L : Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women Metabolism 20:927 1971

IV Sjöström L Smith U Krotkiewski M. & Björntorp P : Cellularity in different regions of adipose tissue in young men and women Metabolism in print Dec 1972

V Björntorp P & Sjöström L : Number and size of adipose tissue fat cells in relation to metabolism in human obesity Metabolism 20:703 1971

VI Sjöström L : Fatty acid synthesis de novo in human adipose tissue I Effects of a high-carbohydrate diet in obesity of different cellular types

VII Sjöström L : Fatty acid synthesis de novo in human adipose tissue II Effects of a high-carbohydrate diet combined with a positive energy balance in obesity

These papers will be referred to by their Roman numerals

The English was corrected by John Kral M A M D

Printed i offset
Elanders Boktryckeri Aktiebolag
Göteborg 1972

C O N T E N T S

INTRODUCTION

The adipose tissue mass of the body depends on the size and total number of the fat cells This simple fact has been known for a long time (Cf 91) but in the absence of adequate techniques adipose tissue cellularity has not been systematically studied until recently Pioneer studies were made by Roh (91) and Bjurulf (5) who studied fat cell sizes in different regions of the body The clinical implications of their studies were limited since the studies were solely made on material from autopsies preventing among other things relating the cellularity of adipose tissue to metabolism The relationship between coronary arteriosclerosis and fat cell size in Bjurulfs work was however a most important finding which indicated the necessity of further work in this field

To study these problems an adequate method for determination of adipose tissue cellularity _in vivo_ was obviously required The percutaneous adipose tissue needle biopsy technique of Hirsch et al (58 60) proved to be a very useful method to obtain adipose tissue specimens in a non-traumatic way which is necessary in research in the clinic Since the available techniques for determination of fat cell size could not be used on needle biopsies new methods were worked out (for review see I) An excellent but expensive osmium fixation technique was described by Hirsch and Gallian in 1968 (59) At the same time that this method was published a simple microscopic technique for determination of fat cell size was in use in this laboratory Comparisons of the two techniques demonstrated a close agreement (I)

A curate estimations of body fat were made possible through the development of under water weighing and isotope dilution techniques (for review see 70 83) Body fat divided by the mean fat cell size obtain d from determinations in one or several parts of the body gave an estimate of the total number of fat cells in the body Studies on adipose tissue cellularity according to these principles were first published by Hirsch Knittle and Salans (62) Later more detailed information has appeared on the total cellularity (III IV V

14 16 24 28 30 33 95) and the local cellularity (IV) of adipose tissue

Obviously the function of any tissue is determined by the cells and not inert substances as for instance calcium salts in bone In adipose tissue this is of particular interest because the inert lipid droplet is located within the fat cell and constitutes most of the volume of the cell and the tissue as a whole In contrast the metabolically active cytoplasm of the adult fat cell only constitutes a small part of the total volume of the cell

The concept of the fat cell as the functional unit and reference basis in in vitro experiments on carbohydrate and lipid metabolism proved to be of considerable value The first such experiments were published in the mid 60-ies by Björntorp et al (8 9 17 63) and Hirsch Knittle and Salans (62 96 97) Later correlations between adipose tissue cellularity and metabolic variables in vivo were reported from this laboratory The most important finding seemed to be the positive correlation between fat cell size and plasma insulin concentration after an over-night fast and/or during a glucose tolerance test found in randomly selected middle-aged men (III 12 16) in obese patients (V) and in patients with endogenous hypertriglyceridemia (15) In obesity (113) and in 19 not specified cases (114) these observations have later been confirmed by other groups

Insulin correlated less strongly to body fat than to fat cell size (V 16) and this interesting observations called for an explanation It is a well-known fact that obesity is associated with a high incidence of decreased glucose tolerance (27 67 87) in spite of abnormally elevated insulin levels (3 27 68 88 97) Several investigations indicate that in vitro large fat cells are less sensitive to insulin than small ones (47 64 94 97 111) One possible explanation for this might be that the hyperinsulinemia of obesity is a compensatory adaptation to a peripheral insulin resistance in adipose tissue as first suggested by Salans Knittle and Hirsch (97) For such an explanation to be valid the total uptake of glucose by adipose tissue should be of such a magnitude that resistance to this uptake would cause an increase of blood glucose resulting in a

concomitant increase of insulin secretion The present work (II VI VII) is a part of the effort made in this laboratory to determine the extent of glucose uptake of human adipose tissue in vitro and in vivo (9 10 11 19)

Since fatty acids are the most concentrated form of energy known in nature a large synthesis de novo of these acids in adipose tissue would be expected to be an important means of storing glucose carbon In several species this is in fact the case (74 86) Since the adipose tissue mass in man is comparatively large it is of interest to investigate the quantitative importance of the fatty acids synthesized de novo in human adipose tissue as possible acceptors of glucose carbon This seems to be of particular interest in conditions with peripheral insulin resistance as discussed above

Because of large discrepancies in available results (for review see II) it was difficult to evaluate the quantitative importance of de novo fatty acid synthesis in human adipose tissue Some authors reported that fatty acid synthesis could reach levels of quantitative importance (29 49) while others reported that necessary enzymes for fatty acid synthesis were virtually absent in human adipose tissue (103-106) It is plausible that these deviating results depended on differences in feeding states types of subjects or assay techniques With whole cell techniques dilution of labelled precursors might conceal a ignificant amount of de novo synthesized fatty acids Subcellular techniques on the other hand usually do not reflect in vivo rates of fatty acid synthesis since among other things such a limiting factor as substrate availability is circumvented

Since most reports on human adipose tissue indicated that fatty acid synthesis de novo in fact is low it was considered of interest to ascertain the maximum overall capacity of the involved enzymes and thus the upper limit of de novo fatty acid synthesis Preliminary experiments in this laboratory indicated that available spectrophotometric assays of one of the individual enzymes in the sequence of fatty acid synthesis were not applicable in human adipose tissue studies and that the crude cytoplasmic assay systems so far used (66 103-106) were not optimal Therefore an assay system (II) was worked

out that determined the incorporation of labelled acetyl units into fatty acids at a rate approaching the maximum overall capacity of the enzymes for the synthesis of fatty acids This assay system was used in experiments with different diets (VI VII) in an attempt to estimate an upper limit for fatty acid synthesis

To sum up the aim of the present investigation was to:

1 develop an adequate method for determination of fat cell size in man

2 investigate adipose tissue cellularity in obese subjects and controls and to search for correlations in vivo between cellularity and metabolism

3 develop an optimal subcellular assay system to determine the maximum capacity of the enzymes for fatty acid synthesis de novo in human adipose tissue and thus estimate the maximum capacity for this synthesis

METHODS

Fat Cell Size

Techniques for determination of fat cell size are detailed in paper I A short summary is given here and some additional information is given in an Appendix

Microscopic techniques A small adipose tissue sample (2 - 15 mg) was fixed in 35 % formaldehyde for 7 minutes The sample was then frozen on the table of a microtome by carbon dioxide snow (I) Lately a tissue freezer (Kryofix H and M, WKF Brandau/Darmstadt West Germany) has been used instead to freeze both the adipose tissue sample (Kryofix H) and the knife (Kryofix M) The new procedure does not influence the quality of the slices and is easier and less expensive to use

A frozen-out slice 200 µm in thickness was microscopically examined in a closed glass chamber containing Ringer s solution A scale divides the eyepiece of the microscope in 100 parts One scale unit corresponds to 2 44 µm The mean fat cell weight from 100 measured diameters could be calculated in two different ways (Methods I:a and I:b) as described in paper I and Appendix In Method I:a the mean fat cell volume was an average of 100 volumes calculated on the separate diameter determinations In Method I:b the mean fat cell volume was calculated from the mean diameter and its standard deviation according to the formula by Goldrick (55) Weights were calculated from volumes using the density of human fat cell triglycerides (70) in both methods For routine purposes Method I:b was used

Osmium fixation techniques With these techniques osmium fixed fat cells suspended in an electrolyte were measured and/or counted with an electronic particle counter A Coulter Counter Model B as well as a Celloscope 302 was employed A Celloscope 302 with a 600 µm capillary tube was used in the final comparisons between the

osmium fixation method and the microscopic method Suspensions of osmium fixed fat cells were prepared according to Hirsch and Gallian (59) with the improvements given in paper I The fat cell size was determined according to Methods II:a or II:b described in paper I and Appendix The calculation procedure of Method II:a was identical to that of method III of Hirsch and Gallian (59) Principally the total lipid weight of a sample is divided by the number of fat cells in that sample Method II:b was a new procedure to determine the mean fat cell volume by measuring with the counter Determinations of the sample wet weight and the ratio of lipid to wet weight were circumvented which is an advantage when only small adipose tissue samples are available

Body Composition

Direct measurements Body height was measured with the subject standing Body weight without clothes was recorded on a scale with a sensitivity of ± 0 1 kg Circumferences were measured with the subject standing In men the maximum waist circumference was recorded during expiration In women the largest gluteal circumference was recorded In order to achieve highest possible reproducibility all measurements were performed by the same person Triceps, subscapular and abdominal skinfolds were measured with a Harpenden caliper (45)

Body potassium was determined either by isotope dilution technique using ^{42}K (exchangeable potassium) or with a whole body counter (total body potassium ^{40}K) Exchangeable potassium was calculated from the specific activity of $^{42}K/^{39}K$ in the urine after 36 hours equilibration of 100 μCi of ^{42}K taken per os This procedure has been described in detail earlier by Lindholm (78) and by Berg and Isaksson (4) Total body potassium was determined in a whole body counter (109)* The whole body counter was calibrated by measuring the counts from subjects before and after administration of known

* Performed by B Arvidsson Ph D at the Radiation Physics Department Sahlgren s Hospital Göteborg, Sweden

amounts of ^{42}K (2) Total body water was essentially determined as described by Lindholm (79) and by Berg and Isaksson (4) Tritium (400 µCi) taken per os was equilibrated for 5 hours The activity of a vacuum destillate of urine from the 6th + 7th hours and 8th + 9th hours was determined and the mean activity of the two samples was used to calculate total body water Close agreement between the activity of the two samples indicated complete equilibration

Calculated body compartments In randomly selected middle-aged men and women (III) body fat was determined anthropometrically The regression equations given below were found to be most accurate up to 30 kg of body fat when comparing with body fat determined with the isotope technique (see below) in other samples of the same population

Men (16):

BF = 0 381 Cw + 0 019 SF - 24 78

(BF is body fat in kg CW waist circumference in cm and SF sum of the three skinfold measurements in mm)

Women (III):

BF = 0 65 Cg - 47 5

(Cg is the gluteal circumference in cm)

In papers IV-VII body fat body cell mass and extracellular water were calculated from the isotope measurements as described by Berg and Isaksson (4)

Adipose Tissue Cellularity

The total fat cell number of the body was calculated by dividing body fat with an estimated average body fat cell weight* calculated from one or several regions

* In this thesis mean fat cell weight indicates the value calculated from 100 fat cell diameters in a slice from one adipose tissue site Average body fat cell weight refers to the estimated mean fat cell weight of the whole body and is obtained as an average of available mean fat cell weights

The local fat cell number was evaluated with a new approach using regional measurements of mean fat cell volume and skin plus adipose tissue thickness The mean fat cell volume was determined with the microscopic technique (I) and the skin plus adipose tissue thickness with a modification (see IV) of the ultrasonic technique described by Booth et al (25)

The calculation of local fat cell number was based on a cylinder with a base surface of 1 mm^2 and a height equal to the skin plus adipose tissue thickness The assumption was made that this thickness was solely made up of adipose tissue since it was not possible to discriminate between the echo originating from the dermis-adipose tissue border and the initial pulse

The "extracellular" volume in the cylinder was found in Bjurulfs diagram relating extracellular space (=non fat cell space) to cell size (6), at the appropriate mean fat cell size The total intracellular fat cell volume of the cylinder was obtained as the difference between the total volume and the extracellular space of the cylinder Local fat cell number was then calculated by dividing the total intracellular fat cell volume of the cylinder with the mean fat cell volume of the region

Blood and Urine Chemistry

When not stated otherwise all analyses of blood were performed on venous blood samples taken after an over-night fast The adipose tissue biopsies were taken after the blood samples

After extraction according to Folch (50) serum triglycerides *were determined as described by Carlson (34) and* serum total cholesterol by the method of Cramér and Isaksson (37) Blood glucose was determined by the glucose oxidase method of Keston (69) as modified by Levin and Linde (75) The "Glox" assay kit (Kabi AB *Stockholm Sweden) was used Plasma insulin was* determined with the radio-immunological method of Hales and Randle (57) using assay kits from Amersham in papers III-V (Insulin Immunoassay kit The Radiochemical Centre Amersham Buckinghamshire England) and from

Pharmacia in papers VI and VII (Phadebas[R] Pharmacia Uppsala Sweden) Urine nitrogen was determined at the Institute of Clinical Nutrition Göteborg Sweden A micro Kjeldahl method was used utilizing a Technicon autoanalyzer with digestor model 1 (Technicon Controls INC Chauncey New York USA)

Adipose Tissue Metabolism

Whole cell technique The incorporation of glucose into CO_2 triglycerides and fatty acids by adipose tissue slices was studied with a conventional technique (VI) Two 200 mg specimens of adipose tissue were incubated in duplicate in 3 ml Krebs-Ringer-Bicarbonate solution (115) (half the prescribed concentration of Ca^{2+}) The gas phase was O_2:CO_2/95:5 The water phase contained 5 5 mM glucose and 15 10^6 cpm [U-^{14}C]glucose When indicated 1000 µU insulin per ml was added

Subcellular technique Fatty acid synthesis de novo was determined in an optimal fortified cytoplasmic assay system (II) measuring the incorporation of acetyl units from acetate and citrate at a rate which probably approached the overall maximum capacity of the present enzymes (VI VII)

Potter-Elvehjem homogenates (90) of adipose tissue were subjected to high-speed centrifugation yielding a fat fraction a soluble cytoplasmic fraction and a sediment of nuclei mitochondria and microsomes Practically all lipogenetic activity was found in the soluble cytoplasmic fraction of the homogenate This activity represented a de novo fatty acid synthesis as demonstrated by TLC avidin inhibition and CO_2-dependence The main products were myristic and palmitic acids

Fig 1 demonstrates the principal of the assay system. Curve I represents C^{14}-acetate (2 5 mM) incorporation at different unlabelled citrate concentrations Curve II shows the incorporation of acetyl units from C^{14}-citrate in the presence of 2 5 mM unlabelled acetate Except for the labelling curve I and curve II are identical Thus,

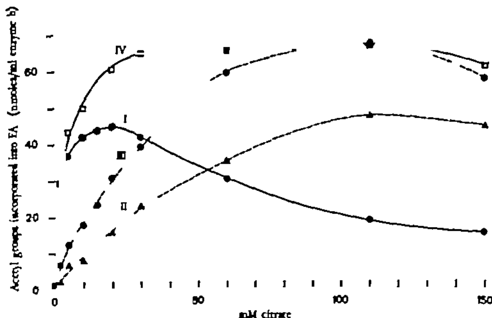


Figure 1 Influence of citrate concentration on [1-C^{14}]acetate and [1-C^{14} 5-C^{14}]citrate incorporation into fatty acids For explanations see text

the sum of curves I and II (=curve IV) shows the total incorporation of acetyl units derived from acetate and citrate at different citrate concentrations Curve III represents C^{14}-citrate incorporation at different citrate concentrations without acetate present in the assay system The results in Fig 1 suggested that at least three different types of incorporation might give equal incorporations of acetyl units viz C^{14}-acetate plus C^{14}-citrate incorporation with 30 mM citrate, C^{14}-acetate plus C^{14}-citrate incorporation with 110 mM citrate and finally 110 mM C^{14}-citrate incorporation without acetate present in the assay system A number of experiments shown in Table 1 however demonstrated that this unfortunately was not the case Although the three types of incorporation gave results of the same magnitude for a given enzyme preparation in individual experiments these results sometimes differed more than could be explained by the error of the method From the experiments in Table 1 it seemed

Tabl 1 The influence of citrate concentration on acetat and citrat incorporati n i to fatty acids

The Roman numerals of the columns correspond to curve numbers in Fig 1 with respect to assay conditions

Exp no	Citrate conc (mM)	Incorporati n of acetyl groups from acetat (I)	itrate (acetat present) (II)	citrate (acetate excluded) (III)	Sum of acetate (I) and citrate (II) incorporation (IV)
		nmol s/ml hour			
1	30	57 1	30 9	67 7	88 0
	110	39 1	67 2	120 9	106 3
2	30	35 2	19 9	48 0	55 1
	110	24 9	26 0	63 4	50 9
3	30	5 4	3 2	4 0	8 6
	110	3 1	3 9	7 2	7 0
4	30	10 1	4 8	8 3	14 9
	110	3 7	12 6	13 8	16 3
5	30	12 6	4 8	15 1	17 4
	110	8 8	17 7	30 4	26 5
6	30	88 6	109 6	93 5	198 2
	110	54 1	128 1	180 4	182 2
7	30	48 5	67 8	55 9	116 3
	110	28 1	72 2	104 3	100 3
8	30	56 2	62 4	122 4	118 6
	110	24 1	61 2	130 1	85 3
9	30	43 3	35 3	50 0	78 6
	110	18 1	44 2	89 1	62 3
10	30	44 2	36 2	47 1	80 4
	110	35 0	51 2	53 9	86 2
Mean±SD	30	40 1±25 6	37 5–34 0	53 2–38 8	77 6±58 4
	110	23 9–16 3	48 4 36 8	79 4±55 8	72 3±52 1

Analysis f variance
all ight group F(7 72) 2 02 n
only the three groups with mean value in box F(2 27)=0 04 n s

possible to conclude that determinations of acetate plus citrate lipogenesis at both 30 and 110 mM citrate revealed the maximum or almost maximum incorporation of acetyl units in most individual cases These incorporations have been used throughout the present investigation Incorporation of 110 mM C^{14}-citrate without acetate present was a fairly good approximation of the maximum capacity but was occasionally much lower

Statistical Methods

The statistical methods are presented in the separate papers (I-VII) Ordinary group comparison t-test pairing design t-test linear regression and analysis of variance have been used Significant F-values have been further analysed with tests according to Scheffe (99) Dunnett (Cf 77) or Tukey (Cf 77) In paper VI a balanced block design of analysis of variance was carried out by an IBM-computer according to the program BMD 02V in Analysis of variance for factorial design (43)

Error of Methods

The standard error of a single determination (38) expressed in % of the mean of 40 - 100 double determinations for different analysis are presented below: Glucose 2 5 %; Insulin (Amersham) 7 1 %; Insulin (Pharmacia) 4 1 %; Triglycerides 2 5 %; ^{42}K 4 5 % (ref 78); total body water 4 %; fat cell diameter 2 5 %; fat cell weight 8 %; acetyl unit incorporation by cytoplasmic assay 3 0 - 14 % for different types of incorporation see paper II

As repeatedly noted in the literature (22 97) the error of methods are large for metabolic human whole cell experiments In the present investigation the errors were: Glucose incorporation into CO_2 27 %; glucose incorporation into triglycerides 20 %; glucose incorporation into fatty acids 24 % The errors calculated on incorporation values expressed per g TG

Fifteen consecutive determinations of serum cholesterol were performed with regular intervals during 6 months on a control serum (Hyland Costa Mesa Calif USA) The mean value of these determinations was 174 1 ± 6 6 mg corresponding to a coefficient of variation = 3 8 % The coefficient of variation for ^{40}K was 1 7 % (ref 2)

In the present investigation all analyses except cholesterol and ^{40}K were performed in duplicate

DISCUSSION OF THE METHODS

Comparison of Different Fat Cell Sizing Methods

When measuring a large number of fat cell diameters a normal distribution is obtained (Cf Fig 5 in paper I) Mean fat cell weight determinations according to Methods I:a and I:b gave almost identical results (Fig 2) The formula for mean fat cell weight determination used in Method I:b is valid only when the cell diameters are normally distributed Thus the strong correlation between results obtained with the two methods demonstrates that counting 100 cell diameters resulted in a distribution sufficiently close to the normal distribution For routine measurements (II-VII) Method I:b has been used

Results with Method II:a and Method II:b correlated closely both when measuring with the Celloscope (r=0 998) and the Coulter Counter (r=0 954) (Fig 7 in paper I)

Since the microscopic methods may be criticised because of the

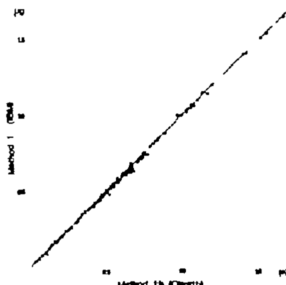


Figure 2 Comparison of the two calculation procedures of the microscopic method for det rmination of fat cell size y 0 993x-0 001 r=0 999 $p(t_b)<0\ 001$ n 273

small number of counted cells and the risk for subjective errors it was necessary to compare the microscopic method with an objective one The osmium fixation techniques were considered to be the most objective ones so far available since large numbers of cells are measured electronically However this does not exclude important subjective errors in the osmium technique since the subjectively determined inflection point of the cumulative curve corresponding to the size of the smallest fat cells in the sample is often indistinct The error of the osmium technique has not been reported by Hirsch and Gallian (59) According to Brook (32) the coefficient of variation of method III of Hirsch and Gallian (=Method II:a in paper I) is 7 5 % This error is of the same order as the error of the mean weight determinations with the microscopic method (8 % paper I)

Even if it is not possible to establish which method is the most objective one there was a close correlation between fat cell weights determined with the microscopic Method I:b and the osmium fixation Method II:a (r=0 960) and the regression line did not differ significantly from the identity line (I) In comparison with the osmium techniques the microscopic method is simpler speedier and much cheaper

Choice of System and Substrate for the Study of Fatty Acid Synthesis de Novo

As was mentioned in the introduction most previous investigations seemed to indicate that fatty acid synthesis de novo in human adipose tissue is of little quantitative importance In order to estimate the maximum capacity of the enzymes for this synthesis in different physiological and pathological conditions an optimal cytoplasmic assay system was developed Mitochondria and microsomes were excluded from the system to avoid β-oxidation as well as chain elongation of fatty acids A crude cytoplasmic system was chosen because the purification of individual enzymes would result in a loss of activity which would be difficult to control thus making quantitative studies uncertain The activity for the synthesis of fatty acids

was almost completely recovered in the soluble fraction of the adipose tissue homogenate (66 Table 1 in paper II) This also seems to be the case in fractionated homogenates of rat adipose tissue (92)

Different radioactive precursors were considered of varying validity as substrates in assay systems set up to measure the maximum capacity of the enzymes for fatty acid synthesis Because of this the following considerations were taken into account:

Labelled acetyl-CoA has often been used as a precursor in subcellular studies of lipogenesis in different species and organs (53 54 82 85 105 106 118) Acetyl-CoA incorporation into fatty acids however requires the presence of some tricarboxylic acid since these acids activate acetyl-CoA carboxylase [E C 6 4 1 2] (for review see 116) The most potent activator is citric acid (82) Citrate however is also an important precursor in acetyl-CoA formation via ATP citrate lyase [E C 4 1 3 8] (for review see 52) If measures are not taken to prevent acetyl-CoA formation from citrate and CoA (obtained by deacylation of labelled acetyl-CoA or malonyl-CoA) an uncontrolled dilution of labelled acetyl-CoA may result making quantitative determinations impossible

Malonyl-CoA is the product of the acetyl-CoA carboxylase reaction This step is commonly considered rate-limiting in different species and tissues (26 36 53 65 82 85 110 118) and thus malonyl-CoA is not an appropriate precursor to study the limitations of the enzymes for fatty acid synthesis The activity of acetyl-CoA carboxylase has been determined by measuring $^{14}CO_2$-fixation from $H^{14}CO_3^-$ (82 106) Labelled CO_2 is presumed to be fixed to malonyl-CoA This seems to be an adequate assumption when working with purified acetyl-CoA carboxylase (82) but not with crude cytoplasmic systems (106) since other carboxylase reactions might occur (119) Furthermore contrary to most earlier reports recent experiments indicate that fatty acid synthetase may have a lower activity than acetyl-CoA carboxylase in adipose tissue in the rat (35) For these reasons measurements of the CO_2-fixation from $H^{14}CO_3^-$ was not considered adequate for the present purpose

Since pyruvate dehydrogenase [E C 1 2 4 1] is an intramito-

chondrial enzyme (98) pyruvate can not be used as precursor in cytoplasmic systems For the same reason none of the glycolytic intermediates preceeding pyruvate can be utilized though they are metabolized in the cytoplasmic compartment (40)

Acetyl(-)carnitine probably is of minor importance as an acetyl-CoA precursor in fatty acid synthesis (31)

The remaining precursors of extra-mitochondrial formation of acetyl-CoA and fatty acids seem to be acetate and citrate The present assay system measured the simultaneous incorporation of acetyl units from these precursors at a rate probably approaching the maximum overall capacity of the present enzymes to synthesize fatty acids de novo In comparison with systems using acetyl-CoA as a precursor the assay system described here depends on two additional enzymes that can possibly be rate-limiting: acetyl-CoA synthetase [E C 6 2 1 1] and ATP citrate lyase

R E S U L T S

Adipose Tissue Cellularity

Body fat average body fat cell sizes and total fat cell numbers in investigations III IV and V are summarized in Table 2 In the table the results in paper V have been divided according to sex In Table 3 results are given from consecutive measurements of fat cell weights in three adipose tissue regions The subjects in Table 3 ranged from slim to severely obese Close correlations were demonstrated between fat cell sizes in the different regions in both men and women

Control subjects In randomly selected middle aged men (n=49) and women (n=58) body fat was larger in women than men but the difference could not be explained in terms of average body fat cell weight or total fat cell number If sick subjects and those on a diet were excluded (Table 1 in paper III) fat cell weight as well as fat cell number correlated positively with body fat in both men and women These correlations were also found in 11 male students (22 - 24 years) (III)

The body weight of middle-aged men at about 20 years of age could be traced from military records Middle-aged men who had increased more than 10 kg in body weight during the last 35 years were compared to those who had kept a steady weight and to the male students The men who increased in weight had more body fat than the other two groups caused by an increased average body fat cell weight There were no differences in fat cell number between the three groups Middle-aged men with a steady weight and male students had similar fat cell weights

In an attempt to explain the well-known differences between the sexes with respect to body fat and subcutaneous adipose tissue thicknes (81 117) adipose tissue cellularity was determined in young male (n=11) and female (n=13) students who had a weight-index close t 1 0 (IV) From determinations of total as well as local

Table 2. Body fa and ad pose ... cellularity in young (II IV) middle-aged (III) and obese ( ) men and women.

		Young		Middle-aged (III)		Obese ( )		p-values*					
		paper IV	III	All	sick and dis ng excluded**								
		( )	( )	( )	( )	( )		( )-( )	( )-( )	( )-( )	(b)-( )	(b)-( )	(a)-( )
				9	45	9							
	Body fa kg	4.8-3	3 4-4.	13.8-5 6	14 13.2	59 0-21 7	19 7	n.s.	005	<0 005	n.	<0.005	0.005
	Average body fa cell weigh ug	0.27-0.	0.44-0 10	0.53-0 28	0.63-0.25	17-0.30	30.4		0 005	<0 005		005	005
	Total ce number 10	.8-1.0	3.1-0 7	3 8-1 4	2.6-0 9	4 9-2.8	13 9	0.10	<0 10	0.005	n.	0.01	0.005
		3		50	54	16							
	Body fat, kg	6-3 6		14.5-5	15.0-4 6	46.4-5 9	100 9		01	<0.005			0.005
	Average body fa ce we ght, ug	0 31-0.07		0.64-0.23	0.64-0. 9	07-0.27	67		0 005	0.005			<0 005
	Total fa ce number 10	4.1 9		3 3 3	3 0- 0	4 6 9	15		0.	n.			.005
Diff men-women	Body fa	001		.05	0.10	10							
	weight	n.s.		n.s.	n.s.	n.s.							
	ll number	0.001		n.s.	n.	n.							

gn fican d fferences between groups analysed with linear contrasts according to Scheff (99). For st tical reasons both group ( ) and ( ) can not be compared with the other groups. Here ( ) ( ), ( ) and ( ) have been combined ( ) substituted for ( ) the results ill be slightly ifferent) as indicated th arrows | indicates ahi from not-s gnifican or almost (10) igni- ficant differences to significan fference | indicates shif from no al non ignificant or ignifican difference to non-significant ifference

** "Sick ...-cases" in paper III excluded.

† Differences between sexes analysed th group comparisons t-tes

Table 3 Mean fat cell weight and intercorrelations in three adipose tissue sites of 75 consecutively examined men and women.

		Men		Women	
Number of subjects		75		75	
Fat cell weight µg:					
range all regions		0 07 – 1 57		0 11 – 1 74	
Hypogastric (mean±SD)		0.46 ± 0 26		0 64 ± 0 34	
Femoral (mean±SD)		0 53 ± 0 27		0 75 0 37	
Gluteal (mean±SD)		0 51 0 26		0 75 0 32	
F(2 222)		1 41		2 56	
p				<0 10	
Correlations		Eqv	r	Eqv	
x	y				
Hypog	femoral	y=0 91 +0 10	0 87***	y=0 96 +0 13	0 89***
Hypog vs	gluteal	y=0 88x+0 10	0 88***	y=0 79x+0 24	0 84***
Femoral vs	gluteal	y=0 83x+0 07	0 86***	y=0 79x+0 16	0 91***

*** p<0 001

fat cell numbers it was demonstrated that the greater body fat of women was mainly caused by an increased fat cell number The average body fat cell weight and the distribution patterns of adipose tissue thickness and local fat cell number were not significantly different between the sexes (IV)

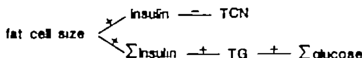
The middle-aged men and women (III) had more body fat than the male and female students (IV) respectively This was explained by larger fat cell sizes in both the middle-aged populations while there were no differences in total fat cell numbers between the groups (IV)

Obese subjects The body fat of the obese subjects (n=37) (V) was about 3 times greater than in randomly selected middle aged controls (III) There was a strong positive correlation between body fat and total fat cell number Contrary to the control groups body fat and fat cell weight did not correlate in the obese groups There were no differences in body fat or adipose tissue cellularity between obese men and obese women (Table 1) On an average the obese subjects had 72 % larger fat cell weights and 52 % more fat cells than

the controls In moderate obesity (25 - 40 kg body fat) increased cell size determined the greater quantity of body fat compared to controls It was suggested that these obese subjects might be separated as a clinical subunit designated hypertrophic obesity With increasing obesity an increasing number of fat cells contributed proportionately more to body fat (V) Generally this hyperplastic obesity was combined with large fat cells Pure hyperplastic obesity appeared to be found only in subjects who were sick or on a reducing diet (Cf Table 1 in paper III)

In vivo correlations The significant correlations between adipose tissue cellularity and metabolism reported in papers III IV and V are summarised in Fig 3 In obese subjects and in middle-aged men fat cell size correlated positively with fasting insulin and with the sum of insulin values during the oral glucose tolerance test

Middle-aged men (n=49):



Middle-aged women (n=23):

no correlations

Obese subjects (n=26):

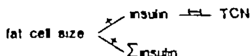


Figure 3 Correlations between adipose tissue cellularity and metabolism reported in papers III-V Correlations are indicated with + and - Signs within brackets indicate trends toward significant correlations (p <0 10)

These correlations were not found in middle-aged women In middle-aged men the sum of the insulin values correlated with serum triglycerides which in turn correlated with the sum of glucose values during the oral glucose tolerance test Almost identical correlation coefficients were obtained when the fat cell size was expressed as diameter surface or volume (not shown in Fig or Tables)

Adipose Tissue Metabolism

Precursors of fatty acid synthesis de novo Contrary to earlier reports by Shrago et al (103-106) the present investigations (II VI VII) have indicated the presence of ATP citrate lyase [E C 4 1 3 8] in human adipose tissue In vitro the capacity of the enzymes for citrate incorporation is at least as large as the capacity for acetate incorporation into fatty acids if optimal conditions are used for both precursors (II VI VII Table 1)

Fatty acid synthesis in adipose tissue in different regions of the body To find out if the fatty acid synthesis in any single region is representative of the adipose tissue as a whole the synthesis of fatty acids was determined in five different regions using the subcellular system Details of the experiments and results are presented in Fig 4

Expressed per g TG the activity of biopsies taken toward the end of the operation (E) was about 15 % lower than biopsies in the same region from the beginning of the operation (e) (e=21 8 $\pm$ 18 0 E=18 2 $\pm$ 15 8 nmoles/g TG mean $\pm$ SD n=4 $p < 0\ 05$ paired comparisons) For this reason the "E values were excluded from further calculations Fig 4 clearly demonstrates that variations within the subjects were much less pronounced than between different subjects There were significant differences between the mean values of all the subj cts except the two with the lowest levels of activity (Fig 4 top section) On the other hand no differences between the different regions could be demonstrated when values from the six patients were pooled In fact the variation between different

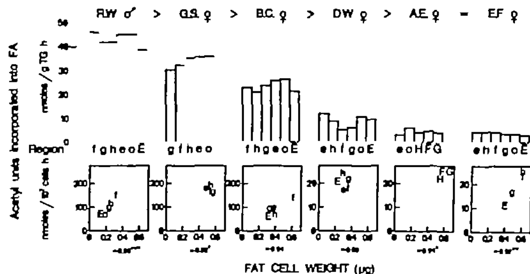


Figure 4 Enzyme acti ity for f tty acid synthe i i different adipose ti sue ite Adipose ti sa bi p i from the femoral (f) gluteal (g) hypogastri (h) pi gastri ( ) and omental ( ) regions were perf rmed in five women and on man in conne tion with cholecystectomy Except for their gallbladd r disease the pati ts were ppare tly healthy They were premedicated with diazepam and tropi The biop ies in each subject were taken i the same order as the stapl are arranged Small l tters ignify that th biopsy wa removed ithin 30 minute f th induction f the narco i ith hexobarbital oxygen nitrous id and succinyl choline Afte these biopsi were taken anae the i wa continued ith fluothane When indicated ev bi y ies were take t the end f the operation (bl ak l tters) afte an verage f 90 170 minut f anae the ia

Incorporation f acetyl unit from [1-C^{14}]acetate and [1-C^{14} 5-C^{14}] itrate was d termined with the cytoplasmi assay system ing 30 mM itrate (Cf "Methods) A ti ity xpre sed per g TG (top tion) or per 10^7 fat cells (bottom section) The results in the top se tion were exami ed with analyse f variance according to two-way lassification with ingl bservation in each e ll (76) Th "E-val were ex luded from calculation There were highly ignificant diff rences between subject [F(5 20) 385 p <0 001] but no difference between different ite [F(4 20) 2 01] The re idual tandard devi tion was 1 4 units hich i equal to 6 9 % f th mean

Produ t moment correl tions ( ) between fat cell weight and tivity per cell are given t the bottom f the figure r calculated on the fi first bi psi † p <0 10; p <0 02; ** p <0 01; *** p <0 001 If E-val are in luded in th calcul tions equal or higher d gree f ignificanc are btained

regions was not larger than the error of the method

Expressed per 10^7 fat cells there were large variations in fatty acid synthesis between different regions However these variations were explained by different cell sizes in different regions A positive correlation between fat cell weight and activity per cell could be demonstrated (Fig 4 bottom section)

In five subjects (RW excluded) regional studies of glucose incorporation into fatty acids in slices were also performed Similar

results were obtained but the intra-individual variations were much larger than in the subcellular assay making the statistical analysis less convincing (not shown in Fig)

Effects of adipose tissue cellularity In the whole cell or subcellular systems there were no significant differences in fatty acid synthesis expressed per g TG between normal hypertrophic hyperplastic or combined hyperplastic-hypertrophic adipose tissue (VI)

Expressed per cell large fat cells showed a trend toward greater whole cell fatty acid synthesis In the cytoplasmic assay system this tendency reached full statistical significance (VI) These findings on whole cell and subcellular fatty acid synthesis in relation to cell size have previously been demonstrated also in fat cells separated into different size classes from the same adipose tissue (20)

Rate-limiting factors in fatty acid synthesis de novo As discussed in papers VI and VII the high ratio of cytoplasmic acetyl unit incorporation into fatty acids/whole cell glucose incorporation into fatty acids calculated on a molar basis indicates a) that the supply of acetyl units and not the capacity of fatty acid synthesizing enzymes is rate-limiting in fatty acid synthesis and b) that the incorporation rate of acetyl units determined by the cytoplasmic assay system may approach the maximum capacity of the enzymes The latter point is supported by the fact that palmityl(+)carnitine does not significantly increase the incorporation of acetyl units into fatty acids (VII)

Using whole cell preparations incorporating glucose and acetate into fatty acids Del Boca and Flatt (41) and Saggerson and Greenbaum (92) demonstrated that the supply of acetyl units is rate-limiting for fatty acid synthesis in rat adipose tissue (see VII) When paper VII was submitted for publication identical experiments had not been performed on human adipose tissue but an additional interpretation of human whole cell data presented by Björntorp et al (19) made it possible to arrive at the same conclusion (VII)

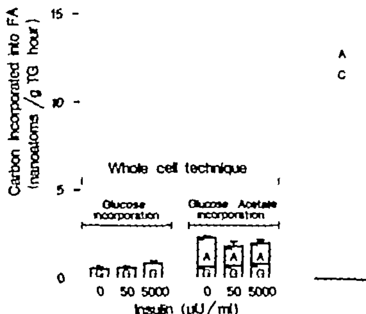


Figure 5 Rate limitation f f tty i4 synthe is Whol cell technique Adipose tissue specimens from man undergoing cholecystectomy were incubated and analysed as described in Methods With 0 and 000 μU insulin/ml triplicate were analyzed ith 50 μU/ml duplicate The l ft group f columns hows the incorporation f carbon from 5 5 mM gl cose (G) (2 10^7 cpm(U-C^{14})gl cose) ith the insulin concentrations indicated Bars represent 1 SD In the right group f columns 5 5 mM gl cose and 5 5 mM acetate were present in the medium In one series f incubations glucose (G) was labell d (2 10^7 cpm(U-C^{14})gl cose) and in another acetat (A) was labelled (8 10^6 cpm (1-C^{14})acetate) The bars withi the column indicate the 1 SD i terval f r incorporation f carbon from gl cose or acetat On SD f the incorporation of carbon fro acetate plus gl cose i indicated at the top f th G A -columns Gl cose and acetat incorporations were paired t random.

Gluco i corporation was insulin sensitive [F(2 5)=17 9 $p < 0$ 01] but not in the presence f cetate The ac tate incorporation was smaller with than without insulin [F(2 5)=41 1 $p < 0$ 005] Without insuli present there wa a trend toward larger gl cose incorporation with than ithout cetate present ($p < 0$ 10 paired comparison) The incorporation f carbon from glucos plus acetate was large than from gl cose len (for i cubati with 0 μU insulin/ml $p < 0$ 001; for 50 μU/ml $p < 0$ 10; for 5000 μU/ml $p < 0$ 01; paired comparisons)

Cytopl smic ass The maximal incorporati f acetyl arbon from acetat (A) pl i rate (C) measured as d cribed in Method

Data from a single experiment with the same design as in the rat experiments (41 92) using human whole cell preparations are shown in Fig 5 These results indicate that the sequence of enzymes active in the synthesis of fatty acids can not be rate-limiting for the incorporation of carbon from glucose alone since the addition

of acetate to the whole cell system doubles the total incorporation of carbon This incorporation however is also far below the enzyme capacity to incorporate acetyl carbon into fatty acids as shown by the cytoplasmic assay (Fig 5 right column) The experiment is in accordance with the high subcellular/whole cell ratios demonstrated throughout papers VI and VII

Effects of high-carbohydrate feeding Effects of a high-carbohydrate-low-fat diet were studied during long term moderately negative (VI) and short term positive (VII) energy balances The glucose incorporation into fatty acids by slices as well as the capacity of the enzymes for fatty acid synthesis increased substantially by both treatments However the higher levels reached during this stimulation (80 nmoles/g TG hour) were of little quantitative importance Glucose incorporation into glyceride-glycerol and CO_2 in slices was not changed by hypocaloric* high-carbohydrate feeding (VI) while hypercaloric feeding increased the incorporation into glyceride-glycerol (VII)

Both of the high-carbohydrate treatments increased serum triglycerides and decreased serum cholesterol while blood glucose and glucose tolerance were unchanged (VI VII) Hypercaloric high-carbohydrate feeding increased plasma insulin (VII) while hypocaloric feeding did not (VI)

Correlations (VI) The correlations between the fat cell size and the enzyme capacity for fatty acid synthesi increased during the course of high-carbohydrate feeding During but not before high-carbohydrate feeding there were several correlations between different whole cell and subcellular activities of adipose tissue During the feeding there were also strong correlations between the amount of fatty acids synthesi ed in adipose tissue and the levels of serum triglycerides The latter correlation was most likely not due to a direct dependence between the two variables

* In this thesis the terms hypo- iso- and hypercaloric refer to the amount of calorie required to maintain a steady weight

Adipose Tissue Cellularity

Determinations The total fat cell number of the body was calculated by dividing body fat with an estimated average fat cell weight Obviously this procedure is inexact

In animal experiments it is comparatively easy to determine body fat by removing defined regions of adipose tissue or by analysing the triglyceride content of the whole animal Body fat determinations in man are of course much more complicated since direct measurements are not possible Instead body fat must be estimated from body density body potassium, tritium space or anthropometric measurements using several assumptions about the composition of the body These approaches can all be criticised (73) but close agreements between different methods (44 51, 71 84 102 120) indicate that several of them may be adequate In the present investigation body fat was calculated as one part of a four compartment system using both potassium and tritium isotopes (4) This procedure may result in smaller errors than calculations based on only one isotope though it can not be proved because of the lack of direct methods for comparisons

A method to determine body fat independent of all assumptions about body composition has recently been published (73) The method which is based on the uptake of highly fat-soluble inert gases may turn out to be excellent for calibration of other methods though it at present seems too complicated for most clinical investigations

While body fat probably can be estimated accurately enough the determination of the average fat cell weight of the body is apparently more complicated To find out if this is at all possible it is necessary to make an analysis of the variations of fat cell sizes within and between different regions of adipose tissue in the body

The variation within one region is rather limited since there is a close correlation between double determinations of the mean fat cell size in different specimens from the same adipose tissue site

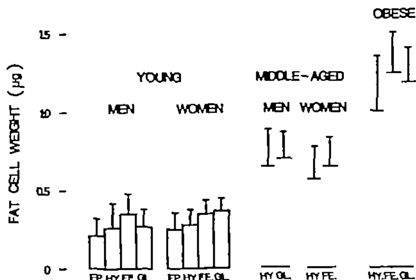


Figure 6 Mean fat cell sizes in different adipose tissue sites of subjects examined in paper III-V The values for the middle-aged women refer to the mean fat cell weight in the hypogastric region of 23 randomly selected women and to the mean fat cell weight of the femoral region in 35 other randomly selected women In middle-aged men (n=15) and obese subjects (n=23) mean values are calculated only on those subjects where complete data from the indicated sites were available

(r=0 99 paper I) The high correlation (r=0 96) between the microscopic (I:b) and the osmium fixation (II:a) method points in the same direction since Method I:b determines only 100 cells from one lobulus while Method II:a determines a large number of fat cells from several lobuli Recently this type of evidence has been found when comparing results from Method I:b with microscopic measurements of collagenase liberated fat cells (112)

The variations in fat cell sizes between different regions have been examined in several types of subjects in the present work The results are summarised in Fig 6 There were no significant differences between the fat cell sizes of the epigastric hypogastric femoral and gluteal regions in young men (IV) of the hypogastric and gluteal regions in middle-aged men (III) or of the hypogastric and femoral regions in middle aged women (III) In young normal women (IV) and in obese subjects (V) the smallest mean fat cell weight

was 20 - 30 % smaller than the largest mean cell weight ($p < 0.05$) These differences would have been still larger if fat cells from adipose tissue in peripheral or intraabdominal regions had been investigated since fat cells in these regions are known to be smaller than in other regions (5 32 56 80 91 93 Cf Fig 4) Theoretically to determine the exact weight of the average fat cell of the body it would be necessary to know the total number of cells in each and every region of the body and the mean weight of the cells in each of these regions This is of course an impossibility However the *differences in mean fat cell weight in different* adipose tissue sites within the different groups of subjects mentioned above are small in comparison with the differences between the average body fat cell weight of these different groups (Table 2 Cf Fig 6) Furthermore there are significant correlations between the fat cell sizes of different sites within different groups of subjects (IV V 5) including correlations between subcutaneous omental and peripheral fat cells (5 56 80) Correlations between different regions in subjects with a wide range of fat cell sizes are very strong in both men and women (Table 3) All these facts indicate that it is appropriate to estimate an average body fat cell weight from mean fat cell weights from a few or with small intra-individual variations even one single adipose tissue site Thus it seems plausible that the total fat cell number of the body determined by dividing the body fat with the average body fat cell weight is an adequate index of the true number of fat cells in the body Since the smaller fat cells of peripheral and intraabdominal adipose tissue were not examined in the present investigations the average body fat cell weight is probably over-estimated and thus the total fat cell number under-estimated

Control subjects An analysis of adipose tissue cellularity in obesity or any other pathological condition requires a comparison with the so-called normal condition Though the distinction between obesity and normality is much disputed the problem usually is circumvented by chosing statistical definitions Disregarding such an obvious limitation of this approach as for instance the fact

that increased weight and obesity are not equivalent phenomena statistically defined borderlines are arbitrary Such arbitrary limits probably have to be accepted however until risk factors and diminished life-expectancy can be taken into account in the diagnosis of obesity

Since there is no generally accepted definition of normal weight and obesity it is not astonishing that the norms for adipose tissue cellularity have not been established In fact so far very little attention has been paid to the reference problem In table 4 results from the few papers providing data on both fat cell size and number have been summarized Investigations reporting only fat cell size are not included (47 48 56 72 80 93 97) The groups have been small (28 61 95) most of the controls have been chosen among the ill (28 33 61) and some of them have not been described with respect to age and sex (61) When age is presented the ranges have often been extremely wide (28 33) The collagenase technique used by Bray (28) can be suspected of selectively rupturing large fat cells since at a body fat of about 20 kg he obtains much smaller fat cells and thus a much higher total fat cell number than is reported in comparable studies (III 33 61) To sum up it seems that the cellularity of the adipose tissue of only 5 healthy subjects within a narrow age range has so far been reported in the literature (95) These subjects served as their own controls in an elegant investigation of experimental obesity performed by Salans Horton and Sims (95)

Obviously it might be hazardous to use the values obtained from these control subjects (28 33 61 95) as normal values when comparing with obesity Table 2 clearly demonstrates how different so-called normal materials might be even when examined by the same technique As is the case in obesity the boundaries of normal adipose tissue cellularity might be better defined when risk factors and diminished life-expectance can be included in the analysis for different parts of the spectrum In the present study the difficult question of normality has been circumvented by comparing obese subjects (V) with a randomly selected population (III Cf Tables 2 and 4) To avoid a large frequency of drop-outs rapid and non-

Table [illegible] [illegible] [illegible] adipose tissue [illegible] larity

[illegible]	Ref	Number of persons	Sex	Age (years)	Clinical data	Method: Body [illegible]	[illegible] weight	Preparation	Determination	Adipose tissue region used for fat weight determination	Body fat (%)	[illegible] (μg)	Total [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	19-78	Coronary artery disease, hypertension [illegible] The rest [illegible] ulcers, duodenal [illegible] pyelonephritis, osteoarthritis, renal [illegible] obesity (body fat 45 kg) [illegible]	[illegible] / Anthrop.	[illegible]	[illegible] / [illegible]	Electron [illegible] / Microscopy	Gluteal / Abdominal	70-80 / 20,3-80	0,6651-0,0874 / 0,74-0,076	[illegible] / [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	20-50	Healthy	Under water weighing	Osmium fix.	Electron	Mean of [illegible], abdominal triceps	[illegible]	0,45-[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Elective surgery	Anthrop.	Osmium fix	Electronal	Site of surgery	[illegible]	0,55-0 [illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	30 or [illegible]	Randomly selected	[illegible]	Processes	Microscopy	Abdominal / Abdominal (50 years) or gluteal (5 years)	[illegible] / 5-5	0,55-0,[illegible] / 0,62-0 [illegible]	[illegible]

[illegible] body [illegible] fat. Body weight not given. [illegible] for [illegible] young men and women see Table [illegible]

traumatic methods had to be used When the investigations in paper III and V were performed no whole body counter was available and the determination of exchangeable potassium required 36 hours of urine collection Since glucose tolerance tests and adipose tissue biopsies also had to be performed it was considered unrealistic to determine body fat with the isotope technique Instead anthropometric measurements were used to determine body fat by means of regression equations High correlations were obtained between anthropometric measurements and body fat determined with isotope technique (III 16) The present reference material (III) demonstrates the associations between body fat fat cell size and fat cell number It is of particular interest that both fat cell number and fat cell weight correlate with body fat in the normal weight range while the correlation between fat cell weight and body fat can not be demonstrated in obese subjects (V 28 61)

No doubt random selection would have been preferable also in the analyses of adipose tissue cellularity in young men and women (IV) However the primary intention of paper IV was not to get control or normal values to compare with different pathological conditions but rather to search for differences in adipose tissue cellularity between normal young men and women Such differences had been suggested in the middle-aged populations (Cf V) and it was considered easier to analyse this question in younger men and women An adequate analysis of these problems however also required examination of the subcutaneous adipose tissue cellularity in different sites The necessary measurements and other determinations were considered impossible to accomplish in randomly selected samples Instead young healthy subjects with a weight index (present weight/ideal weight in relation to height) close to 1 was chosen in an attempt to measure the mean of the population Unfortunately this selection probably resulted in samples which are not representative for the general population the subjects being too tall and too slim. This is particularly obvious when these young highly selected men (IV) some of which were athletes are compared with medical students (III) which had not been preselected (Cf Table 2) However the selection probably allowed conclusions about the differences

in adipose tissue cellularity between the sexes (IV)

Obese subjects Obesity was arbitrarily defined as beginning at a body fat of 25 kg The subjects were patients sent for general examinations at the First Medical Service Sahlgren s Hospital

There were no significant differences in adipose tissue cellularity between obese men and women (Table 2) Considering this and the limited number of subjects examined obese men and women were pooled for the analysis of relationships between body fat fat cell size and fat cell number When the obese groups were compared to randomly selected subjects, women were chosen since most of the obese subjects were women Also there were no statistically significant differences in fat cell size or number between the randomly selected men and women This comparison made it possible to suggest a hypertrophic type of obesity associated with moderately increased body fat and a more severe hyperplastic obesity the latter as a role combined with fat cell hypertrophy

In a simultaneous report Hirsch and Knittle did not present any obese subjects where hypertrophy contributed more than hyperplasia to body fat when comparing with a reference material (61) This can be explained by the fact that too few cases with moderate obesity were examined While 21 of the 35 subjects in the present investigation (V) had a body fat below 50 kg in Hirsch s material the corresponding figures were 2 of 20 subjects (61) If the same analysis as in Fig 3 of paper V is performed on Hirsch s data the y-axis will be located to the right of the intersection of the fat cell number line and the fat cell size line Thus subjects with cellularity characteristics falling to the left of this y-axis were not studied by these authors

<u>Correlations</u> In the present investigations positive correlations have been demonstrated between fat cell size and insulin levels in middle-aged men (III) and obese subjects (V) The lack of correlation in the comparatively few middle aged women (III 23) is not necessarily a female characteristic since positive correlations between fat cell diameter and fasting insulin in young women can be demonstrated

by using primary data of study IV ($r = 0.60$ $p < 0.05$) Furthermore most of the obese subjects in paper V were women The association between fat cell size and insulin level has also been found in hyperlipidemic women (15) Though recent preliminary reports have confirmed the correlations between fat cell size and insulin levels (113 114) more studies are necessary in subjects of both sexes representing different physiological and pathological conditions So far the correlation has not been possible to demonstrate in maturity onset diabetes (18)

A further analysis of the relationship between obesity and plasma insulin levels in a larger group of subjects (108) preliminary indicate that weight change is a most important factor for the concentration of plasma insulin In obese subjects increasing in body fat over a period of about nine months the insulin levels were higher than in the obese subjects whose weights were unchanged during this time The lowest insulin values were found in obese subjects who had decreased in weight These findings might indicate that the correlations between insulin levels and fat cell size are associated with changes in the fat depot During weight gain increases in fat cell weight and insulin levels might be parallel phenomena whether mutually dependent or not The fact that physical training lowers insulin levels to normal in hyperinsulinemic obese subjects in spite of maintained or increasing body fat (13) suggests that increased fat cell size is not of primary importance for the development of hyperinsulinemia during weight gain in sedentary subjects Whatever the mechanism for the association between fat cell size and insulin level the stability of body weight apparently has also to be taken into account as an important variable

The Quantitative Importan e of Fatty Acid Synthesis for Lipid Storage and Gluco e Uptake in Human Adipose Tissue

The ratio of fatty acid synthesis de n vo in man can be increased several times by high-carbohydrate feeding (VI VII) The levels reached by timulation are low in comparison with the fatty acid synthesis in adipose tissue of several other species (74 86) The

small amounts of fatty acids synthesized in whole cell systems (VI VII and review in I) could partly be explained by the dilution of labelled precursor by unlabelled compartmentalized precursor pools While the maximum capacity for fatty acid synthesis of the enzymes involved is several times greater than the registered incorporation of glucose (VI VII) or glucose plus acetate (p 27) into fatty acids by whole cell preparations, it is still low from a quantitative point of view During hypercaloric high-carbohydrate feeding the maximum capacity of the enzymes incorporating acetyl units into fatty acid on an average reached only 80 nmoles per g adipose tissue triglyceride and hour Since there are no significant differences in this enzyme activity expressed per g triglyceride in different adipose tissue sites (p 25) or in different cellular types of adipose tissue (VI) it seems reasonable to make extrapolations from single adipose tissue preparations to the total adipose tissue mass Then 80 nmoles/g adipose tissue TG hour corresponds to a synthesis of 0 06 g palmitic acid/kg body fat 24 hours Assuming that 1 molecule of glucose produces 2 acetyl units this synthesis would require only 0 17 g glucose/kg body fat 24 hours These are maximum values which indicates that fatty acid synthesis de novo in human adipose tissue most likely is low even in extreme conditions such as carbohydrate feeding of hyperinsulinemic subjects with a severe obesity With a body fat of 50 kg only 3 g of palmitic acid per day would be synthesized corresponding to a glucose consumption of about 9 g As discussed in paper VII these maximum figures are in good agreement with in vivo studies (11)

Apparently other fatty acids must play a major role in the process of lipid storage in human adipose tissue This view is supported by the fact that lipoprotein lipase in human adipose tissue (for review see 89) has 100 to 1000 times larger capacity to make fatty acids available for storage than the enzymes involved in the synthesis of fatty acids de novo Since the fatty acid synthesis also is low in human liver (100 101 107) it seems likely that exogenous fatty acids are of major importance for the storage of fatty acids in adipose tissue This suggestion is supported by the fact that the pattern of fatty acids in adipose tissue tends to be similar to that in food (1 7 39 58)

G E N E R A L S U M M A R Y

The aim of the present investigation was to study the cellularity of human adipose tissue in relation to sex age and obesity Furthermore, the fatty acid synthesis de novo in adipose tissue was studied in relation to cellularity obesity and different diets These studies required new methods:

1 A microscopic method for the measurement of fat cells in frozen-cut adipose tissue was developed and compared to the electronic method of Hirsch and Gallian The correlation between the methods was high (r=0 96) Some improvements were made on the electronic method and a new calculation procedure was suggested The microscopic method was simpler faster and much cheaper

2 Using the method for fat cell size determination and ultra sound measurements a procedure was developed to estimate the local number of fat cells in perpendicular adipose tissue cylinders

3 A subcellular assay system was developed to determine the overall maximum capacity of the enzymes involved in fatty acid synthesis de novo in human adipose tissue by measuring the simultaneous incorporation of labelled acetyl units from acetate and citrate The activity of enzymes to incorporate acetyl units into fatty acids was almost exclusively recovered in the cytoplasmic fraction of the fat cell Several conditions were crucial for the assay system Linear incorporations with respect to time and enzyme concentration were attained The main products of the system were myristic and palmitic acids

With these methods the following results were obtained:

4 Fat cell size and number were determined in randomly selected middle aged men and women In both sexes fat cell size as well as number correlated with body fat Women had more body fat There were no differences in fat c ll size or number between

young men and middle-aged men who had maintained a steady weight since they were 20 Middle-aged men who had increased in weight had more body fat than young men due to larger fat cells

5 Young women had more body fat than young men due to an increased total and local number of fat cells The distribution patterns of subcutaneous fat and local" fat cell number in four examined regions were similar in men and women

6 In obese subjects fat cell number correlated with body fat while fat cell size did not In moderate obesity (25 - 40 kg of body fat) the greater amount of body fat compared to controls seemed to be caused by an increased cell size (hypertrophic obesity) With increasing obesity an increasing number of fat cells contributed proportionately more to body fat This hyperplastic obesity generally was combined with large fat cells

7 Significant positive correlations were found between the fasting insulin concentration and fat cell size in obese subjects and middle-aged men The sum of the insulin values during the oral glucose tolerance test and fat cell size also correlated

8 In vitro the capacity of the enzymes to incorporate citrate was at least as large as the capacity for acetate incorporation into fatty acids if optimal conditions for both precursors were used The supply of acetyl units and not the capacity of the enzymes synthesizing fatty acids is probably rate-limiting for fatty acid synthesis de novo in human adipose tissue

9 Expressed per g TG the overall activity of the enzymes involved in fatty acid synthesis was not different in different adipose tissue sites or in normal hypertrophic hyperplastic and combined hyperplastic hypertrophic adipose tissue Large fat cells were metabolically more active than small cells especially during carbohydrate feeding

10 Fatty acid synthesis and the enzymes necessary for this synthesis adapt to carbohydrate feeding Even the maximum levels reached are however of little quantitative importance for fatty acid storage or for glucose uptake in human adipose tissue

A C K N O W L E D G E M E N T S

My sincere thanks to

Associate professor Per Björntorp M.D who has initiated the present study and has been my teacher and co-worker He has encouraged my work and followed the investigation with the most stimulating and constructive criticism

Professor Lars Werkö M D who has given me excellent laboratory facilities and working conditions and has followed the experiments with keen interest

Professor Björn Isaksson M.D and Mrs Ivy Lilimägi at the Institute of Clinical Nutrition who provided help and advice with the nutritional parts of the investigation

Professor Lars Svennerholm, M.D who has supplied laboratory facilities for gas chromatographic studies at the Department of Biochemistry and has given valuable criticism during the process of the work

Associate professor Anders Gustafson M D for generous help with all serum lipid electrophoreses and for most fruitful discussions

Professor Esbjörn Carlström Ph D who initiated me in biometry and who has provided most valuable statistical advice throughout the whole investigation

Miss Birgitta Hedmark for her never-failing sense of duty and painstaking and skilful technical assistance in all phases of my work

Mrs Karin Winqvist B A for computer service and data revision

Dr John Kral M.A M.D for having corrected my English and for stimulating discussions

My coworkers and to all colleagues at the Surgical Departments of Sahlgren s Hospital who have willingly performed adipose tissue biopsies especially Drs Erling Cahlin M D Jan Holm M.D Jane Jönsson M.D John Kral M.A M D and Associate professor Tore Scherstén M.D who performed most of the biopsies

I also want to express my sincere thanks to Miss Majvor Karlsson Mrs Kerstin Ahlqvist Miss Ann Ahlström Mrs Mariann Delin Mrs Agneta Eggers Miss Lonica Hadders Mrs Anita Hovden Miss Inga Hvass Mrs Ebba Hästbacka Miss Aino Johansson Mrs Kia Jäderberg Miss Ann-Britt Ljunggren Mrs Anita Samuelsson and Miss Anne-Marie Sjöquist for valuable help and skilful assistance The nurses and staff at ward 33 Sahlgren s Hospital are gratefully acknowledged for all their help in the nutritional experiments

I am very grateful to Miss Agneta Orrhult for her devoted and excellent secretarial help

I am grateful to AB Bofors for economical support and supplying Vivasorb

This investigation was supported by grants from the Swedish Medical Research Council (Grants no B69-19X-251-06A B70-19X-251-07B B71-19X-251-08) Swedish Nutrition Foundation Swedish Diabetes Association *Nordisk Insulinfond and Tore Nilssons s Fund for Medical* Research

R E F E R E N C E S

1 Albutt E C & Chance G W Plasma and adipose tissu fatty aids of di betic children long term corn il di t J Clin Inve t 48 139 1969

2 Arvidsson B Sköldborn H & Isaksso B Clinical application f high-ensitivity whol body count Dige t f the 3rd int rnational conf rence f medical physics in l ding medical eng ing Ed R Kadefors B I Magnusson and I Petersen Gothenburg pape 41 2 1972

3 Bagdad J D Bierman E L & Po t D J Th ignificance f basal insulin level in the evaluation f the in uli response to gl cose in di beti and non-di beti subj t J Clin Inve t 46 1549 1967

4 Berg K. & Isaksso B Body compo ition and nutrition f cho l children with cerebral palsy In Adapti rebral Palsy f Body Compo ition Nutrition and Physical Working Capac ty t School Age by Berg K. p 41 suppl no 704 A ta Paediat Scand 1970

5 Bjurulf P Athero cl ro i and body build with spe ial ref rence to ize and number f subcutane us fat cell A ta Med Scand. Suppl 166 1959

6 Bjurulf P Extracellularrummet per mm^3 d subcutana f ttva en ho manniska Communication from the Department f Anatomy University f Lund Sweden

7 Björntorp P Polyunsaturated f tty ds in man Scand J Cli Lab Inve t 12 Suppl 52 1960

8 Björntorp P Studier av human f ttva vid be itas Svenska föreningen för invärtesmedi i Oct 16 1965 (N rd Med 75 722 1966 abstr)

9 Björntorp P Studi adipose ti su from bese patients ith without diabete mellitu II Basal and insulin- timulated gl cose metabolism. A ta Med Scand 179 229 1966

10 Björntorp P Th ff t f insulin in vitro human adipose tissue from normal and diabeti subj t A ta Med Scand 181 389 1967

11 Björntorp P Berchtold P Holm J & Larsson B The gl cose uptake f human adipose ti su in be ity Europ J Cl Inve t 1 480 1971

12. Björntorp P Berchtold P & Tibbli G Insulin secretion in relati to adipose tissue in men Diabete 20 65 1971

13 Björntorp P de Jounge K Sjö trd L. & Sullivan L. The ffect f physical training on insulin producti in be ity Metabolism 19 631 1970

14 Bj rntorp P Grimby G Sanne H. Sjöström, L Tibblin G & Wilhelmsen L Adipose ti sue fat cell ize in rel ti to metabolism in weight tabl physi cally active me Horm. Metab Re 4 182 1972

15 Björntorp P Gustafson A & Persson B Adipo tissu f t cell i and numbe in relation to metabolism in endogenous hypertriglyceridemia. Acta Med Scand 190 363 1971

16 Björntorp P Gu tafson A & Tibblin G R latio hip between adipose ti su cellularity and carbohydrate and lipid metabolism in randomly l ted popu-l tio In Atheroscl rosi Proceedings f th cond internati nal symposium" Ed by R J ne Spri ger-Verlag Berlin p 374 1970

17 Björntorp P & Hood B Studi f adipose tissue from bese patients with or without diabet mellitus I R lease f glycerol and free f tty ids Acta Med Scand 179 221 1966

18 B Örntorp P Jensson A & Bercht ld P : Adipose ti sue ell la ity in maturity on t diabete mellitus A ta Med Scand 191 129 1972

19 Björntorp P Schersté T & Gottfri s A Effects f glucose infusion on dipose ti sue lipogene i in man Acta Med Scand 183 565 1968

20 Björntorp P & Sjö trö L.: The composition and metabolism in vitro f dipose ti su f t ll f diff rent i Europ J Cli Inv t 2:78 1972

21 Björntorp P & Sjö tröm, L.: F t cell i and number in dipos tissue i relation to met bolism Israel J Med Sci 8 320 1972

22 Björntorp P & Ö tman J Human adipos ti sue Dynamics and regulation Ad Metabol Disord 5 277 1971

23 Blehm C Personal communication

24 Bonnet F Ge selin L Chantrain J & Cent rre J : Adipo cell number and siz in normal and t se hildren Rev Eur Etud Clin Bi l 15:1101 1970

25 Bo th R.A D Goddard B A & Paton A : Measureme t of fat thi kne in man: a comparison f ultra ound Harpend calipers and lect ical cond ti ity Brit J Nutr 20:719 1966

26 Bo t Y Abraham S & Chaik ff I L Localizati f the block in lipogen i resulting from feeding f t J Bi l Che 238 1266 1963

27 Bo hell B R Chandali H B Krei berg R A & Roddam R F Serum insulin in obe ity and di bete mellitus Am J Clin Nutr 21 1419 1968

28 Bray G A : Measurement f subcutaneous f t cell from bese patient Ann Int Med 73 565 1970

29 Bray G.A Lipoge i in human adipo ti sue: some ff t f Nibbling and Gorging J Cli Inve t 51:537 1972

30 Bray G A & Gallagh T F Jr R gul tory besity i man Cli R 18:537 1970 b t

31 Bres l E & Brend l K The rol of carnitine a d carniti acyltran fera i bi l gi l acetyl tions and f tty id synthe i J Bi l Chem 241:4092 1966

32 Bro k C G D Comp ition f human adipo ti su from deep and subcutaneous it Brit J N t 25 377 1971

33 Brook C G D Lloyd J & Wolf O H R l tion b tween age of onset f obe ity and i and number f adipo ll Brit Med J 2:25 1972

34 arl L A Det rmination f serum glyce id A ta Soc Med Upsalien 64 205 1959

35 Chakraberty K & Leveill G A A tyl CoA carboxylas and f tty acid synthetase a tiviti in liver and adipose ti su f meal f d rat Pro Soc Exp Biol Med 131 1051 1969

36 Chang H -C Seidman I Te bor C & Lan H.D Liver acetyl CoA carboxylas and f tty id synth tas rel tive ti iti s i th ormal tat and i heredi tary be ites Bi hem Biophy Re Commun 28 682 1967

37 Crand K & Isak o B An evaluation f th Abeerell method f the d t rmination f t tal serum h l terol Scand J Clin. Lab Inve t 11 213 1959

38 Dahlb g C tati tical method f medical and bi l gical tud ts Geo ge Allen & Unwi Lo do p 12 1948

39 Dayto Ha himoto S & Pearce M.L. Adipo ti su linol ic id crit ri f i re ce to modified di t J Lipid R 8 508 1967

40 De Duve C Wattiaux R & Baudhuin P Di t ibution f yme between sub-cellular fractio i animal ti su In Adv E zymol R E Nord (ed) Inter-sc ien Publ In New Y rk p 291 vol 24 1962

41 Del Boca J & Flatt J P Fatty acid synthe i fro gu cos and acetate and the control f lipogene i i adipose ti sue Europ J Bi he 11 127 1969

42 Diem K & Lentner C (eds) Documenta Geigy Scie tifi Tabl 7th d J R Geigy S.A Ba le Switze land p 712 1970

43 Dixo W J (ed) BMD biomedical compute programs University f Calif rni Pre Berk ly and Lo Angel 1968

44 Durni J V G A & Rahaman M,M The asse sment of the amount f f t in the human body from measureme ts f skinf ld thickn Brit J Nut 21:681 1967

45 Edwards D A W Hammond W H, Healy M J R Tanner J M & Whit hou R H : De ign and accuracy f calipers f me suring subcutan ou ti sue thi kne Brit J N tr 9 133 1954

46 Engelhardt A Ori , F A Li bermei te H. A Jahnke K : Size lipid and enzyme content f isolated human adipocyte in relati to nut itional tat Diabetologi 7 51 1971

47 Engelhardt A Kasperek R Li bermei t H A Jahnk K Studi on gl cose tilization and insuli respon iven f human subcutaneous adipo ti sue i be and non-obese subj t Horm Metab R s 3 266 1971

48 Faulhaber J D Petruzzi E H Ebl H & Ditschuneit H I vitro U te such-unge über den Fettstoffwechsel is li rte men hli her Fettzell n i Abhängigk it vo den Zellgrö se Di durch Adrenalin i duzi rte Lipolyse Horm Metab R 1 80 1969

49 Fe l r A Beck J C & Rubinstei D Fa tors ff ting lipid synthe i i human adipose tissu in vitro Metabolism 16 438 1967

50 Fol h J Ascoli I Lees M Meath J A & LeBaro F N Preparati f lipid extract fro brai ti su J Bi l Che 191 833 1951

51 Forbe G B & Levi A H. Total sodium pota ium and chloride in ad lt man J Clin Invest 35 596 1956

52. Fo ter D W & Srere P A Citrate leavage enzyme and f tty acid synthe i J Bi l Chem 243 1926 1968

53 Ganguly J Studi the mechanism f f tty acid synthe is VII Bi synthesi f fatty acid from malonyl CoA Bi chim Biophy Acta 40 110 1960

54 Gibson D.M. Titch ner E B & Wakil S J Studi s on the mechani f f tty acid synthesis V Bicarbonate requirement for synthe i f long hain f tty id Bi chim Biophy Acta 30 376 1958

55 Goldrick R B Morphol gical change in dipocyte duri g f t depositi and mobilization. Amer J Physi l 212 777 1967

56 Goldrick R B & McLoughlin, G M. Lipolysi and lipogene i from glucose i human fat cell f diff rent i J Cli Inve t 49 1213 1970

57 Hal C N & Randle P J Immunoa say f insuli with insulin antibody precipi tate Lancet I 200 1963

58 Hirsch J Farquhar J W Ahren E H J Pet son M L & Stoffel W Studi f adipose ti sue in man microtechnique f sampling and naly i Amer J Clin Nutr 8 499 1960

59 Hirsch J & Gallian E. Method f th d termination f dipo e ll size i man and animal J Lipid R 9 110 1968

60 Hirsch J A Goldrick R B Serial tudie on th metabolism f human adipose ti sue I Lipogene is and free fatty id uptak and releas in small spirated sample of subcutaneous fat J Clin Inve t 43 1776 1964

61 Hirsch J A Knittl J L : Cellularity f be and nonobese human adipose ti sue Fed Proc 29:1516 1970

62 Hirs h J Knittl J L A Salans L B Cell lipid content and cell number in b se and nonobese human adipose tissue J Clin Inve t 45:1023 1966 abstr

63 Hood B A Björntorp P : Studi on adipose tissue from bese pati t with or without diabete mellitus III Transformati n f U-^{14}C-acetat and 1 ^{14}C-glycerol into carbon dioxide and lipid Acta Med Scand 179 349 1966

64 Horton E S Danf rth E Sims E A H A Salans L B : Correlation f forearm mu l and adipo e ti sue metabolism in besity bef re and after weight 1 Clin Re 20 548 1972 b t

65 Howanitz P J A Levy H R Ac tyl-CoA carboxylas and itrate 1 vage nzyme i rat mammary gland Bi him Biophy Acta 106:430 1965

66 Jac b I K : Fatty acid synthe i in cell free preparations f human adipo ti sue Bi che Biophys Acta 70 751 1963

67 J hn H J A summary f findings i 1100 gl cos tol rance timations Endocrinol 13 388 1929

68 Karam J H Grodsky G M A Forsham, P H : Exce sive insulin respo se to glucose i b subject measured by immunochemical assay Diabete 12 197 1963

69 K to A Abstr f Papers 129th Meeting Amer Chem So p 31C 1956

70 Key A A Brožek J Body f t in adult man Phy iol gical review 33 245 1953

71 Kul ish R Fainstei L A And reso E C Correlation of potas ium-40 concentration and f t-free l an co tent f hams S ience 127 338 1958

72 Leonhardt W Han f ld H Sohn id r H A Hall r H Human adipocyt volumes Ma imum size and correlation to weight index in maturity onset diabet Diabet logi 8 287 1972

73 Le C T Dent b S A Mark fsky J Use f i d pendent me surement f body f t to evaluat overweight and und rweight Metabolism 26 792 1971

74 Lev ill G A Adipo ti sue metabolism influe ce f periodi ity f ating and di t compo ition Fed Pro 29 1294 1970

75 Levin K A Li d S Det rmination f glucose i blood cerebrospinal fluid and ine ith a ew gl co e-oxidas reagent J Swed Med A 59 3016 1962

76 Li C C I trod ti to Experimental Stati ti Mc Gra Hill Book Company p 64 1964

77 Li C C I trod tion to Experiment l Stati tics M Graw-Hill Book Company p 418 1964

78 Li d.ol B Body ll mas during long-term rti on tre tment in asthmati subj cts A ta Endocrinol (Kbh) 55 702 1967

79 Li dholm B Body ll mas du ing long-term tre tme t ith cortisone and anaboli teroid i thmati subj t A ta Endocrinol (Kbh) 55 222 1967

80 Li h H J ..ail ..andhef P A Braun teine H C terzu hungen an isoli rter men hli t ll vere hi den F ttgewebe regionen I Be i hunge i en rel ve K rpergewi ht l ..ellvolumen Kli W chr 48 1549 1970

81 Ljunggren H Se diff rence in body compo iti In "Human Body Compo ition J Brozěk (ed) Pergamon Pre Oxford p 129 1965

82. Martin D B & Vagel P R The mechanism f tricarboxyli acid cy 1 regul tion f fatty acid synthe i J Bi 1 Chem. 237 1787 1962.

83 Moore F D Olesen, K.H. McMurray J D Parker H.V Ball M.R & Boyden C M. The body cell mas and it supporting environment Philadelphia Saunders 1963

84 Myhre L.G & Kessler W V Body density and potas ium 40 measurement f body compo ition as related to age J Appl Physi 1 21 1251 1966

85 Numa S Matsuhashi M. & Lynen F Zur Störung der Fettsäuresynthese bei Hunger und alloxan-Diabete I Fettsäuresynthese in d r Leber normaler und hungernder Ratten Biochem. Z 334 203 1961

86 O'Hea E K. & Leveill G.A. Significance f adipose ti su and live as it f f tty acid synthe is in the pig and the ffi i cy f tilization f various substrat for lipogenesis J Nutr 99 338 1969

87 Paullin J E & Saul H C A study f the glucose tolerance test in the bese Southern Med J 15 249 1922

88 Pearly M. & Kipni D M. Plasma insulin respons to glucos and tolbutamid f normal weight and bese di beti and non-diabeti subj ts Diabete 5 867 1966

89 Persson B Schröder G & Hood, B Lipoprotei lipase activity in human adipose ti sue Assay method Relations to the serum triglyceride level in normolipid emi population The ffect f thyl chlorophenoxy isobutyrate Atheroscle 16 37 1972

90 Potter V R. & Elvehjen, C.A. A modifi d method for th study f tissue oxidations J Bi 1 Chem 114 495 1936

91 Reh H Di Fettzellgrösse beim Menschen und ihre Abhängigkeit vom Ernahrungszustand Virchow Arch Path Anat Re 324 234 1953

92 Saggerson E D & Greenbaum, A L. The regulation f triglyceride synthesis and fatty acid synthe i in rat epididymal adipose tissue Effects f altered di tary and hormonal conditions Biochem. J 119 221 1970

93 Sailer S Sandhof F Li oh, H.J & Braunsteiner H Grö se der Fettzellen und relative Körpergewicht in verschi denen Regi en de Menschen Wien M Inn. Med 50 374 1969

94 Salans L.B Horton E S & Sims E Influence f fat cell i and di tary carbohydrate intak on adipose tissu insulin nsitivity in adult besity Clin Re 18 463 1970 bstr

95 Salan L.B Horte E.S & Sims E.A H. Experimental be ity in man Cellular character f the adipos tissue J Clin Inve t 50 1005 1971

96 Salans L B Knittl J L & Hirsch J The rol f adipose cell enlargement in the carbohydrat intoleranc f human be ity J Clin Invest 46 1112 1967

97 Salan L.B Knittl J L. & Hirsch J The rol f adipose cell ize and adipose ti sue insulin sensitivity in th carbohydrate intolerance f human be ity J Clin Inve t 47 153 1968

98 Sanadi D R. Pyruvate and α-ketogl turate oxidation enzyme In "The Enzyme P D Boyer H Lardy & K Myrbäck (eds) Academi Press New Y rk p 307 vol 7 1963

99 Sch ff H.A The analysi f variance J hn Wiley & Son In New York p 66 1959

100 Scherstén T Nilsson Sv Cahlin E & Jildero B Synthesi f phospholipid and triglyceride in human liver II Influence f preoperative fru tose load Scand J Clin Lab Invest 26 399 1970

101 Soherstf T Nil son v & Jönsson J : Hepatic lipogene is in two case ith i sulin-producing tumor f the pancreas A ta Med .cand 190 353 1971

102 Sheng H P & Huggins R A Direct and indirect measurement of total body wat in the growing beagl Proc .o Exp Biol Med 137:1093 1971

103 Shrago E Glennon J.A & Gordon E S Enzyme studi in human liver adipose ti sue Nature 212 1263 1966

104 Shrago E Glennon J.A & Gordon E S Studie on enzyme concentrati and ad pti in human liver and adipose tissue J Clin Endocrinol 27:679 1967

105 Shrago E Glennon J A & Gordon E S Comparative spects f lipogene i in mammalian ti sue Metabolism 20 54 1971

106 Shrago E Spennetta T & Gordon E S : Fatty id synth i in human adipose tissu J Biol Chem. 244 7761 1969

107 Sjö trö L : Unpubli hed bservation

108 S ö trö L & Björntorp P To be publi hed

109 Sköldborn H Arvid son B & Andersson M.: A new whol body monitoring l bora t ry In Proceedings f th Sixth Internati nal Nordi Meeting o Cli ical Phy i Suppl 313 A ta Radi l p 233 1972

110 Smith S Easter D J & Dil R. Fatty id biosynthesi 3 Intracellular it of enzyme in lactating-rabbit mammary gland Biochim. Biophys Acta 125 445 1966

111 Smith U Eff t f cell siz on lipid synth is by human adipose ti sue in vitro J Lipid Res 12 65 1971

112 Smith U Sjö tröm L. & Björntorp P Comparison between two method to d termin human adipose cell ize J Lipid R in pri t 1972

113 t rn J Hollander N & Hirs h J Adipose cell size and immunoreactive insulin level i bes and normal weight adults Fed Pro 30 194 1971 b tr

114 Stern M. Ol fsky J Farquhar J & Reaven, G R l tionship between f t cell i and i sulin re i tance in vivo Cli Res 20 557 1972 bstr

115 U breit W W Burri R.H & tauffer J F Manometri techniqu Burge Publi hing Co Minn poli Minn USA p 149 1959

116 Vagel P R. Alberts A W & Marti D B tudi o th mechani f activation of acetyl-CoA carboxylase by itrate J Bi l Chem. 238 533 1963

117 Vagu J Boy r J J belin J Nicolino C & Pi to C Adipomuscular rati i human subje t In Phy i p thol gy f Adipose Ti sue J Vague & R M. Denton (ed) Excerpta Medica Found ti Amst rdam p 360 1969

118 Wi land O Neuf ldt I Numa S & Lynen F : Zur törung der Fett inresynth t i Hunger und Alloxandi b tes Biochem Z 336 455 1963

119 Wood H.G & Utte M.F The rol of CO_2 fixati in metaboli Essay in Bi hem. 1 1 1965

120 Woodward K Trajill T Sehn h R L. & And rs n, E C C rrel tion of total b dy potas ium with body wat r N ture 178 97 1956

APPENDIX

for calculations of fat cell size

A Microscopic Techniques

Method I:a Assuming that the measured cells are spheres the mean fat cell volume $\bar{v}$ can be calculated from the measured diameters (μ) d_i as the average of all separate cell volumes using an IBM computer:

$$\bar{v} = \frac{\sum_{1}^{100}\left(\frac{\pi \; d_i^3}{6}\right)}{100} \quad \mu^3$$

The mean fat cell weight $\bar{w}$ *is calculated in the same way using* the density of human fat cell triglyceride (0 915 g/ml) (1) to convert the individual volumes to weights:

$$\bar{w} = \frac{\sum_{1}^{100}\left(\frac{\pi}{6} \; d_i^3 \; \frac{0\,915}{10}\right)}{100} \quad \mu g$$

Method I:b With the aid of the average $\bar{d}$ of 100 cell diameters and its standard deviation s $\bar{v}$ and $\bar{w}$ are calculated using an Olivetti computer (Programma 101) according to the formulae (2):

$$\bar{v} = \frac{\pi (3 \; s^2 \; \bar{d} + \bar{d}^3)}{6} \quad \mu^3$$

$$\bar{w} = \frac{\pi (3 \; s^2 \; \bar{d} + \bar{d}^3)}{6} \; \frac{0\,915}{10^6} \quad \mu g$$

1 Keys A & Brožek J : Physiological Reviews 33:245 1953
2 Goldrick R B : Amer J Physiol 212:777 1967

If desirable the mean fat cell surface $\bar{s}$ can be calculated with a similar formula (1):

$$\bar{s} = \pi (s^2 + \bar{d}^2) \quad \mu^2$$

B Osmium Fixation Techniques

The arbitrary volume (V) of particles measured in an electronic particle counter (Coulter Counter Model B or Celloscope 302) is:

$$V \geq \frac{D}{A \quad I} \qquad 1)$$

where D = setting of discrimination
A = amplification
I = aperture current

The true volume (v) and diameter (d) of particles can be written:

$$v \geq V \quad K_v \qquad 2)$$

and

$$d \geq \sqrt[3]{V} \quad K_d \qquad 3)$$

where K_v and K_d are constants

The counter is calibrated by using particles (corn pollen) with a known mean diameter ($\bar{d}$) The settings of D A and I at which 50 % of these particles are counted is considered to correspond to the known mean diameter value $\bar{d}$ Using the values of D A and I and equations 1) - 3) it is possible to calculate K_v and K_d:

$$K_v = \frac{\pi \quad \bar{d}^3}{6} \quad \frac{A \quad I}{D} \qquad 4)$$

1 Zinder J : J Lipid Res 12:91 1971

$$K_d = \bar{d}\ \sqrt[3]{\frac{A \quad I}{D}} \qquad 5)$$

When values of K_v and K_d are known formula 2) and 3) can be used for calculation of the true volume (v) and its diameter (d) respectively from any combination of D A and I The constants must be determined for each individual counter or capillary tube

For each sample the number of cells per ml suspension are determined in quadruplicate at different settings corresponding to a geometrical series of volumes By increasing the sensitivity of the counter the detectable volume decreases with a factor of $\sqrt{2}$ in each step of this series

Coincidental passage through the capillary tube is corrected for according to Wales and Wilson (1) One and occasionally two dilutions of the suspension are performed while counting Dilution factors are obtained by determining the number of counts at the same settings before and after dilution From these determinations a cumulative curve of fat cell volumes or diameters is constructed Calculation of the mean fat cell size is then performed by (one of) the following two procedures:

Method II:a The mean fat cell weight ($\bar{w}$) expressed as the weight of the mean lipid content (µg) is calculated according to the formula:

$$\bar{w} = \frac{\left(\begin{array}{c}\text{wet weight of sample}\\ \text{for osmium fixation } (\mu g)\end{array}\right)\left(\begin{array}{c}\text{ratio of lipid}\\ \text{to wet weight}\end{array}\right)}{(\text{cells per ml}) \qquad (\text{ml of suspension})} \qquad 6)$$

This calculation procedure is identical with that of method III of Hirsch and Gallian (2)

Method II:b The number of counts per ml between all adjacent settings (ΔN) is calculated as well as the corresponding mean arbitrary volumes ($\bar{V}$) Since the V values constitute a geometrical series which decrea ed with a factor of $\sqrt{2}$ in each step the $\bar{V}$ values form a similar series The product $\Delta N \quad \bar{V}$ represents the total arbitrary volume of all cells in 1 ml suspension between two adjacent settings Thus $\Sigma \Delta N \quad \bar{V}$ is the total arbitrary volume of all cells in 1 ml suspension The mean arbitrary fat cell volume ($\bar{V}$) is obtained by

1 Wales M. & Wilson J W : Rev Scientif Instr 32:1132 1961
2 Hirsch J & Gallian E : J Lipid Res 9:110 1968

dividing the total arbitrary volume with the number of cells constituting it that is the number of cells per ml suspension (N):

$$\bar{V} = \frac{\sum \Delta N \quad V}{N} \qquad 7)$$

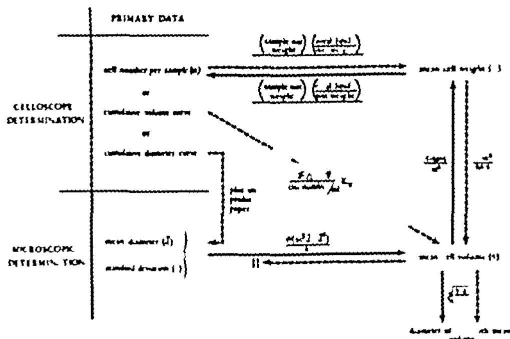
The true mean volume ($\bar{v}$) of the fat cells is obtained from the calibration of the counter (Cf formula 2)):

$$\bar{v} = \bar{V} \quad K_v \quad \mu^3 \qquad 8)$$

The mean fat cell weight ($\bar{w}$) is calculated from the volume using the density of human adipose tissue triglycerides:

$$\bar{w} = \frac{\bar{v} \quad 0\ 915}{10^6} \ \mu g \qquad 9)$$

Interrelationships between different fat cell characteristics are shown below



Acta Medica Scandinavica

Supplementum 546

Respiratory and Circulatory Investigations in Obstructive and Restrictive Lung Disease

By
Senefro K Gabriel

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw kr 170 incl. postage.
Other countries Sw kr 200 incl. postage.

Chief Editor

Professor Jan G Waldenström MD
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Riddargatan 12 B
S-114 35 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26 Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell Informationsindustri AB
S-751-81 Uppsala, Sweden

From the Department of Clinical Physiology
Karolinska Sjukhuset, Stockholm Sweden

Respiratory and Circulatory Investigations in Obstructive and Restrictive Lung Disease

By
Senefro K Gabriel

CONTENTS

INTRODUCTION

The trend towards extensive industrialization and the creation of modern industrial societies have contributed to the increase of chronic non-specific lung disease and occupational lung diseases. Chronic obstructive lung disease, or chronic bronchitis/emphysema, is becoming the most prevalent chronic lung disease in the United States and the mortality attributed to this complex has increased sixfold in a period of 10 years (40). In Great Britain 10 % of death among men between 40—65 years are caused by chronic bronchitis (40). In most countries the increased incidence of this complex and of the occupational lung diseases is becoming a significant factor also in physical disability. In consequence the diagnosis of these diseases is becoming important. However, the problems and difficulties of radiological diagnosis and the discrepancy between radiological and pathological changes demand an elaborate evaluation of pulmonary function to assist in deciding on medico-legal problems.

A large number of analytical methods have been developed, aimed in the first place at determining the pathophysiological changes of respiratory diseases. In the course of time the number and complexity of these methods have increased considerably. The use of pulmonary function tests in the clinical investigation of patients with lung disease has proved to be of a high diagnostic and therapeutic value, and there has therefore been an increased demand by clinicians for these tests. In order to be able to meet these requests and apply the tests to as large a number of patients as possible, it seemed to be of importance to restrict the number of methods in use.

The aim of the present study was to evaluate some of the current methods in order to know which of them have the best practical discriminatory value and to allow an accurate quantitation of physiological derangements. It was also desired to get further information about some of the pathophysiological changes, which will be also reported.

The material for the present study is composed of patients with chronic obstructive and restrictive lung disease. Different methods were applied and a comparison was made for each method. On the basis of this comparison a suitable investigation procedure has been proposed.

CHAPTER I

MATERIAL AND METHODS

Material

The material comprised patients with chronic lung disease referred to this laboratory for routine cardiopulmonary functions tests. The aim in the selection was to collect two groups of patients functionally classified into A) patients with chronic obstructive lung disease, B) patients with chronic restrictive lung disease. Patients with primary heart disease or pneumonectomy were excluded. The following criteria were applied

1) *Clinical criteria.* a) *Chronic bronchitis* History of chronic productive cough for a minimum of three months per year and for at least two years (American Thoracic Society) (1)

b) *Bronchial asthma* was diagnosed whenever the patient had a history of acute attacks of dyspnea and wheeze.

c) *Emphysema.* The diagnosis was based on the following radiological criteria 1 hyperaeration of the lungs, 2. flattened diaphragm, 3. reduced vascular shadow In some patients the radiological diagnosis was combined with clinical and physiological findings.

d) *Pulmonary fibrosis and pleural thickening* The diagnosis was based on clinical, radiological and laboratory investigations.

2) *Functional criteria.* For functional classification the material was divided into two groups.

A) *Obstructive lung disease* FEV % $<x-1$ SD where x is the predicted normal value according to Berglund *et al.* (3) In three patients (Nos. 5, 9 and 16) FEV % was between $x-1.5$ SD and $x-2$ SD in all other patients FEV % was $<x-2$ SD. No regard was paid to the vital capacity

B) *Restrictive lung disease* Vital capacity (VC) <80 % of the predicted normal value and normal FEV %. Cases 8, 12, 13 16 and 17 had a decreased FEV % as well. These patients were included in the group, as they had predominantly restrictive impairment as judged from the spirometric data.

Group A with obstructive lung disease included 24 patients, 16 men and 8 women, with one or more of the following diseases chronic bronchitis, emphysema or asthma. Seven patients (Nos. 1 4 9 10 17 18 and 19) had old healed pulmonary tuberculosis as well. However the radiological evidence of the disease was minimal and the patients' complaints were not related to it. In the 5 asthmatic patients the disease had extended over many years, and was associated with chronic bronchitis and/or emphysema. Except for case 24 they had had at one time or another a positive skin allergen test.

Group B with restrictive lung disease included 17 patients, 12 men and 5

women. Eight patients had pulmonary fibrosis of various etiology and nine had pleural disease. In eight patients the pleural disease was secondary to old pulmonary tuberculosis, of whom 4 had pronounced pleural thickening, 3—7 cm and 4 minor pleural thickening of these 8 patients 6 had been treated with artificial pneumothorax and two with thoracoplasty In one patient (No. 11) the pleural thickening was secondary to influenzal empyema. He also had a kyphotic deformity of the thoracic spine. For descriptive purposes the term pleural restriction will be used in referring to these patients. The main clinical diagnoses are given in Tables I—II Chapter II

Methods

Total amount of hemoglobin (THb g) was determined by the alveolar carbon monoxide method according to and described by Sjostrand (32 33) *Hemoglobin concentration* (Hb g/100 ml) was measured spectrophotometrically (16)

Blood volume (BV 1) was calculated from THb and hemoglobin concentration of fingertip blood and corrected for the systemic difference between the total body hematocrit and peripheral vessel hematocrit using the factor 0 91 For review see 6)

Heart volume (HV ml) was determined in the prone position according to Larsson and Kjellberg (22 with the modification that the X ray tube was inclined 30 caudally 21

Static spirometry Total lung capacity (TLC and its sub[illegible] tal capacity VC [illegible] RV functional residual capacity FRC [illegible] ratio of residual volume to total lung capacity (RV/TLC %) and the ratio of functional residual capacity to total lung capacity (FRC/TLC %) — were determined in sitting position using a closed circuit helium dilution spirometer (14) (Kifa, Stockholm) Normal values were predicted according to Grimby and Soderholm (12) All values were converted to BTPS using the factor 1 1

Dynamic spirometry (1 BTPS) Forced vital capacity (FVC) forced expiratory volume in one second (FEV_1) FEV %

($\frac{FEV_1}{VC}$ x 100) forced inspiratory volume in one second (FIV_1) maximal voluntary ventilation at a respiratory rate of 40 (MVV_{40}) and maximal voluntary ventilation at a free respiratory rate (MVV_{free}) were determined with a Bernstein spirometer (Kifa Stockholm) (4) Normal values were predicted according to Berglund *et al* (3)

Orthostatic test Heart rate was determined in the supine position after 10 minutes rest and after 8 minutes standing erect from an ECG record.

Exercise tolerance test The exercise tolerance test was performed according to Sjostrand (31) and Wahlund (36) on an electrically braked cycle ergometer (15) in sitting and supine position on two different occasions, starting as a rule with 200 kpm/min and increasing the work load stepwise at the end of 6 minutes. Heart rate was determined every two minutes at each work load. The respiratory rate during one minute was determined by auscultation during the third minute of each work period All patients were urged to continue the

work test until disabling symptoms or signs appeared. The rate of work at a heart rate of 130 beats/min (W_{130}) was determined by inter- or extrapolation using an approximately linear relationship between the heart rate and the work load. No regard was paid to the presence of steady state.

The rate of work at the exercise breaking point (W_B). This measure is the same parameter as W_{max} suggested by Strandell (35). In lung disease, however, maximal performance may be affected by external factors, which is why this term was preferred. It was calculated as the highest work load the patient could sustain for 6 minutes with the addition of the load for the next non-completed period after recalculation to refer to 6 minutes. Example: A patient completes the first work period of 6 minutes at a rate of work of 300 kpm/min, while in the next period he interrupts the test after two minutes at a work load of 600 kpm/min: his W_B will be equal to 300 (work load of the first period + 2/6 × 300 (work load of the second period) = 400 kpm/min. The breaking point heart rate (HR_B) was taken as the highest heart rate recorded during exercise. All heart rates were determined from ECG records.

Diffusing capacity of the lung for carbon monoxide ($D_{L_{CO}}$, ml/min/mm Hg) was determined by a steady-state technique according to Filley *et al.* (10) with correction for CO capillary back pressure according to Linderholm (24). $D_{L_{CO}}$ was calculated according to the equation $\frac{\dot{V}_{CO}}{P_{A_{CO}} - P\bar{c}_{CO}}$ where $\dot{V}_{CO}$ is the carbon monoxide uptake, $P_{A_{CO}}$ the alveolar carbon monoxide tension, $P\bar{c}_{CO}$ the carbon monoxide tension in the pulmonary capillaries. The measurements were performed during steady-state exercise on a cycle ergometer in the sitting position. The work load was selected individually from previous exercise tolerance tests to increase the heart rate to ≥ 120 beats/min. Fractions of gases F_{O_2} and F_{CO_2} were analysed by the Haldane technique. Gas volumes were measured with a spirometer. Carbon monoxide in gas phase was analysed with a Hopcalite CO meter (Stålex) as described by Linderholm and Sjöstrand (23). The carbon monoxide content in blood was determined by the method described by Linderholm, Sjöstrand and Söderström (25). The carbon monoxide capacity of blood was taken to be equal to 1.34 ml/g Hb.

The reproducibility of measurements of $D_{L_{CO}}$ during exercise in this laboratory is between 7.2—6.4 % (18, 19).

Alveolar ventilation ($\dot{V}_A$, l/min) and alveolar oxygen tension were calculated from the alveolar gas equation. *Physiological dead space* (V_D, ml) was calculated using the Bohr's equation and assuming the alveolar carbon dioxide tension to be equal to the arterial carbon dioxide tension (9, 28). The respiratory valve had a dead space of 40 ml at rest and 50 ml during exercise.

Oxygen uptake ($\dot{V}_{O_2}$, ml/min), *carbon dioxide elimination* ($\dot{V}_{CO_2}$, ml/min) and $D_{L_{CO}}$ were expressed at STPD.

Total ventilation ($\dot{V}_E$, l/min), $\dot{V}_A$ and V_D were expressed at BTPS.

The veno-arterial shunt (Q_{va}) was measured according to Berggren (2) during 100 % oxygen breathing in supine position until the nitrogen concentration in the expired air reached a level of 2 %. The calculation was made according to the equation

$$\frac{Q_{va}}{Q} = \frac{C_{cO_2} - C_{aO_2}}{C_{cO_2} - C_{\bar{v}O_2}}$$

Where C_{cO_2}, C_{aO_2} and $C_{\bar{v}O_2}$ were end-capillary, arterial and mixed venous blood oxygen contents and Q was the systemic blood flow.

The arteriovenous oxygen difference was taken to be 4 volumes %. In our laboratory shunt values up to 7 % of the cardiac output are considered normal with this technique.

Arterial oxygen tension (Pa_{O_2}, mm Hg) was determined with a Clark electrode.

Arterial carbon dioxide tension (Pa_{CO_2}, mm Hg) was measured with a glass electrode according to Severinghaus. Instrumentation Laboratory Inc. blood gas analyser model 113.

pH was determined by the Astrup ... (illegible)

Oxygen saturation (Sa_{O_2}) was determined ... spectrophotometrically ... [illegible]

[illegible]

Intravascular pressures were measured with Elema differential transformer transducers (EMT 490 A). About half of the material was recorded on an Elema "Mingograf" recorder and the other half with Medec strain gauge transducers (30—145) and an U.V. recorder (Oscillofil, Siemens). Mean pressures were obtained by electrical integration (time constant 1.0 second). The pressure reference level was the midthoracic point of the anteroposterior diameter at the sternal insertion of the fourth rib. *Cardiac output* (Q) was measured according to the direct Fick method. The *pulmonary vascular resistance* index (PVR) was calculated as the difference between the pulmonary artery mean pressure and the pulmonary wedge pressure divided by the cardiac index (C. I.) (cardiac output divided by body surface area). *The systemic vascular resistance* index (SVR) was measured as the brachial artery mean pressure divided by the cardiac index. Expired air was collected in Douglas bags, usually for 8 minutes at rest and 3 minutes during exercise. *Gas volumes* were measured with a spirometer. Expired gas was analysed for oxygen and carbon dioxide by the Haldane technique.

Errors for a single determination estimated from duplicate determinations and the reproducibility of the method are the same as reported in other studies from this laboratory (7, 20).

Statistical Methods and Computer Programmes

I. Statistical methods.

S... calculations were performed ... (Snedecor 1959, 34). Dif...

ferences between regression lines were tested according to Hald (1960) (13). The following probability levels of significance were used:

$p \leq 0.001$ highly significant (***)
$0.001 < p \leq 0.01$ significant (**)
$0.01 < p \leq 0.05$ prob. significant (*)

II *Computer programmes*
Diffusing capacity and ventilation were calculated on an IBM 7044 computer. Correlation matrix and multiple regression analysis were performed with the IBM 1130 computer at the computer centre at Karolinska Institute. Other computations were performed at this department with Olivetti, Electronic desktop computer programma 101.

Definitions, Symbols and Abbreviations

Definitions, symbols and abbreviations used were mainly as recommended by Gandevia and Hugh Jones (11) and Pappenheimer *et al.* (27).

Procedure

All patients were hospitalized during the period of examination, during which routine clinical and laboratory investigations were made. Functional studies started with a determination of total amount of hemoglobin, blood volume, heart volume, two exercise tolerance tests, one in sitting and the other in supine position on two separate occasions, ECG, phonocardiogram and spirometry. Ventilation, diffusing capacity and shunt studies were performed on a separate day, as was also the heart catheterization. The procedures used during the measurements of diffusing capacity and in heart catheterization are reported in chapters IV and V respectively.

Comments

The methods adopted in the present investigations are well established, though their application in the present studies might be subject to criticism. For instance the calculation of $P_{A_{O_2}}$ from the alveolar air equation leads to an error in the presence of uneven ventilation/perfusion, as an arterial alveolar CO_2 tension difference may exist. This results in underestimation of $P_{A_{O_2}}$ and other related variables. Also Mellemgaard (26) has shown that the method of Berggren leads to over-estimation of the veno-arterial shunt in patients with maldistribution of ventilation. However it is assumed that the error which might be introduced will be reflected proportionally in both groups and thus for comparative purposes, it will not significantly influence the results expected.

References are given in the next chapter.

CHAPTER II

LUNG VOLUMES, VENTILATORY CAPACITY and CIRCULATORY DIMENSIONS

Results

Clinical and anthropometric data are given in Tables I—II Spirometric data are

T M I Clinical and anthropometric data in group A 4 patients, 16 men and 8 women with

Subject no.		Sex	Age yrs	Height cm	Weight kg	B.S.A. m²
1	L A.	M	5	168	51.5	1.59
2	K F	M	6	1 4	60.5	1 75
3	H F	M	63	163	66.5	1 7
4	E.E.	M	64	174	69.5	1.83
5	W.K.	M	48	184	69 0	1.9
6	R K	M	40	181	74 0	1.93
7	S J	M	53	176	77 0	1.91
8	W.M	M	63	177	85.5	2 0
9	C F	M	67	1 8	54 0	1 70
10	H M	M	61	17	53 5	1.68
11	F E.	M	69	1	3 0	1.89
1	A G	M	55	165	55 0	1 60
13	J L.	M		185	56 0	1.80
14	A F	M	4	161	56 0	1 .0
1	A W	M	74	17	69 0	1 8
16	P F	M	(	161	49 5	1 54
1	S F	F	13	160	54 0	1 5
1	F K	F	7	161	5 0	1 60
19	I A	F		150	55 0	1 48
20	B W	F	1	161	0	1.53
1	A F	I	3	163	57 0	1 61
	I I	F	48	1	44 0	1 40
3	A S	F	1	16	41 0	1 40
4	L H	I	0	1 4	71 0	1 77
Men						
—			)	173	63 9	1 8
SD			11 6	03	11 0	0 15
R ge			3	163 18	4 85 5	1 54 — 0
			1	1	16	16
W en						
—				1 1	3	1 54
SD				4 0	6	0 1
R ge			4	1 1 4	41 1	1 40 1 77
				8	8	8

given in Tables IV—VI
chronic obstructive lung disease.

Total hemoglobin g	Blood volume l	Heart volume supine ml	Clinical diagnosis
595	5 0	610	asthma + chronic bronchitis
700	5.8	985	emphysema
601	4 8	750	emphysema
472	4.9	640	chronic bronchitis + emphysema
568	4.8	760	emphysema
562	4 4	890	chronic bronchitis
720	5 6	860	emphysema
736	5.3	1020	asthma + chronic bronchitis + emphysema
477	4.5	650	emphysema
438	3.8	503	emphysema
523	3.8	645	emphysema
465	3 3	1145	chronic bronchitis + emphysema + secondary polycythemia
606	4.0	810	emphysema + cardiosclerosis + secondary polycythemia
423	3 4	596	chronic bronchitis + emphysema
700	5.5	805	chronic bronchitis
444	4 0	435	emphysema
488	3.9	575	asthma + emphysema
498	4.2	550	chronic bronchitis + emphysema
415	3.5	—	asthma + emphysema
408	3.3	695	chronic bronchitis + emphysema
385	3 2	680	asthma + chronic bronchitis
347	3.2	525	chronic bronchitis
381	3.9	—	emphysema + fibrosis
566	5 6	730	asthma + chronic bronchitis
564	4.6	737	
107	0.78	193	
423—736	3.3—5.8	435—1145	
16	16	16	
436	3 9	626	
74	0 80	86	
347—566	3.2—5 6	5.5—730	
8	8	6	

T ble II Clinical and anthropometric data in group B. 17 patients, 1 men and 5 women, with

S bject no.		Sex	Age years	Height cm	Weight kg	B.S.A. m²
1	H B	M	60	173	57.0	1 70
2	E E.	M	4	18?	68 0	1.90
3	R.U	M	49	170	64.0	1 75
4	V.S	M	61	167	6.0	1 70
5.	J.S	M	48	171	74.0	1.8
6.	B T	M	37	181	53.5	1 75
7	G.Z.	M	0	176	64.5	1.80
8	E.O.	M	49	182	69.5	1.90
9	E.P	M	50	177	82.5	2 00
10.	C.M	M	67	183	87.0	10
11	L.H	M	77	162	62 0	1.6
12	A R.	M	5	173	79 0	1.93
13	C.J	F	34	168	79 0	1.89
14	S H	F	40	154	65 5	1 62
15	H B	F	49	157	54.5	1.54
16	A D	F	50	166	51.5	1 60
17	I P	F	65	161	6 5	1 68
Men						
			7	174 8	68 9	1.81
SD			10 56	6 65	10.26	0 14
Ra e			4 —77	16 —183	53 5—87.0	1.6 —2 10
			1	1	1	1
Women						
			48	161.	63 8	1 67
SD			11 6	5 89	10 12	0 15
Range			34—65	1 4—168	51.5—79 0	1.54—1 89
			5	5	5	5

T e III A thropometric data differences between group means.

Varia	Sex	Group A	Group B	P
Age ye rs	M	60	7	—
	I	53	48	—
H m	M	173 5	174 8	—
	I	1 .0 1	161	—
W	I	1 9	63 9	—
	I	53	63 8	—
B.S A.		1	1 84	—
		1	1	—

chronic restrictive lung disease.

Total hemoglobin g	Blood volume l	Heart volume supine, ml	Clinical diagnosis
487	4.2	504	left pl. thickening + lobe resection and thoracoplasty
653	4.4	502	left pl. thickening + right thoracoplasty
655	5.7	646	pulmonary fibrosis (ch. interstitial fibrosis)
600	5.5	990	pulmonary fibrosis (ch. interstitial pneumonia)
628	4.9	850	pulmonary fibrosis (silicosis)
563	6.3	610	right pleural thickening
477	4.7	805	pulmonary fibrosis (silicotuberculosis)
610	6.6	600	bilateral pleural thickening
780	6.4	885	pulmonary fibrosis (asbestosis)
734	5.8	970	left pleural thickening
608	4.9	—	left pleural thickening + left contracted lung
829	6.6	790	left pleural thickening + left contracted lung
594	4.6	770	left pleural thickening + left contracted lung
565	4.5	680	pulmonary fibrosis (pulmonary muscular hyperplasia)
362	3.2	650	pulmonary fibrosis + chronic bronchitis
418	3.8	430	pulmonary fibrosis (post irradiation)
534	4.1	620	right pleural thickening
635	5.5	741	
106	0.75	177	
477—829	4.2—6.6	502—990	
12	*12*	*11*	
495	4.0	626	
100	0.57	125	
362—594	3.2—4.6	430—770	
5	5	5	

Variable	Sex	Group A	Group B	P
Total hemoglobin (g)	M	564	635	—
	F	436	495	—
Blood volume (l)	M	4.6	5.5	<0.01
	F	3.9	4.0	—
BV/kg (ml)	M	72.5	81.0	—
	F	72.5	63.6	—
Heart volume, supine (ml)	M	757	741	—
	F	626	626	—

T ble II Spirometric data in group A. For symbols, see methods.

Subject no.		Sex	TLC	VC		RV	FRC
				l	pred.		
1	U A.	M	5.25	3.58	70	1.67	3 23
..	K.E.	M	11 10	4.29	89	6 81	8.90
3	H.F	M	6 77	3.6	85	3 15	3.98
4	E.F	M	6 73	2.8	60	3 41	4 17
5	W.A.	M	9 43	..46	41	6 97	8.33
6	R.K	M	6 48	3.9?	71	..5(	3 83
	S J	M	6 80	3 91	6	81	3 91
8.	W.M	M	6 ?	86	?	4 06	4 6
9	G F	M	3 75	.67	53	3 18	4.39
10	H M	M	8 05	6(	3	3 41	6 70
11	E F	M	7 4(	3.30	1	4 16	5 03
1	A (	M	5 27	08	47	3 19	3 89
13	J I	M	9 33	3 41	68	5.9?	7 1
14	A F	M	6 01	1 91	41	4 07	4 8?
15	A W	M	7 11	4.21	95	3.20	4 6
16	I F	M	??	6(	65	3.33	4 47
17	S E	F	4 72	1 90	37	82	3 49
18	F K	F	5 18	08	63	3 10	3 77
1?	F A	F	3 67	41	95	1 6	11
??	B W	F	4 68	1 91	5	7	3 70
1	A F	F	4 1	1 60	49	2 91	3 ?(
	F P	F	4 10	33	7	1 77	..00
3	A S	F	4 (1	1	4	3 09	3 31
1	L.H	F	5 11	39	6	55	3 1
Men 16		$\bar{x}$	7 14	3 15	6(	4 00	5 11
		SD	1 63	0 74	15 50	1 5	1 69
		Range	5 1 11 10	1 91 1 ??	41 ?	1 6 6 81	3 3—8 90
W e 8		$\bar{x}$	4 58	01	65	31	3 1
		SD	0 ?0	0 33	16 01	0 67	0 51
		Range	3 6 5 18	1 5 39	4 ?	1 ? 3 10	11 3 77

Lung volumes and ventilatory capacity

Group A Stat ve olumes I (TTPS II tal (a men and w n was significantl l n d p I normal l w) II nr apac e RV w g l and n n 161 % and in women 181 of predicted values ($p < 0.001$). FRC was increased in both sexes and in men averaged 1?2 % ($p < 0.05$) and in women 131 % ($p < 0.001$) of predicted values. The RV/TLC % ratio averaged in men nd women 55 % and the FRC/TLC 71 these values being igni

RV/TLC %	FRC/TLC %	FVC	FEV_1	FEV %		FEV_1/FIV_1	MVV_{40}	MVV_{free}
				%	% pred.			
32	6	3 11	1.98	59	74	0 76	49	57
61	80	2.61	1.32	34	49	0.61	32	39
47	59	3.85	1.95	48	68	0.56	68	97
55	67	2.12	0.80	36	54	0 47	20	17
74	88	3.91	2.34	60	81	0.86	58	71
40	59	4 45	3.00	66	86	0.71	92	11
42	58	3.52	1.50	38	53	0.44	68	56
59	69	2.00	0 85	33	49	0 47	29	27
55	76	2.90	1 45	50	75	0 67	39	58
67	83	1 75	0 70	29	42	0.39	19	19
56	67	2.65	1 10	32	48	0.32	41	43
61	74	1 45	0.70	35	49	0 32	25	26
63	76	1.85	0.80	25	39	0.27	29	30
68	80	1.50	0.95	44	61	0.68	15	16
43	64	4 10	1 45	35	54	0.45	62	81
56	75	2 80	1 60	54	81	0.54	43	46
60	74	2.09	1.21	58	73	0.59	31	34
60	73	1 10	0.58	30	39	0 45	19	24
34	57	2.20	1 16	53	71	0 62	34	39
59	79	1 75	0.65	33	42	0.65	16	16
65	72	1 40	0 70	50	65	1.06	14	19
43	71	1.90	1 45	64	80	0.85	46	69
67	77	1.08	0 70	46	59	0 61	26	26
50	61	1 73	0.95	37	47	0 42	23	16
55	71	2.79	1 41	42	60	0,53	43	50
11 43	9 28	0.97	0 66	12.35	15.14	0 17	21 63	28,87
32—68	58—83	1 45—3 91	0.70—3 00	21—71	25—66	0 27—0 86	15—92	16—112
55	71	1 66	0.93	46	60	0 66	26	30
11 46	7 63	0.43	0 32	12.18	15.34	0 21	10 63	17.65
34—67	57—79	1.08—2 20	0.58—1 21	30—64	39—80	0 42—1.06	14—46	16—69

ficantly high in both sexes ($p < 0.001$)

Dynamic lung volumes (l BTPS) Mean FVC was slightly lower than mean VC in both sexes, the difference being non-significant in men ($p > 0.05$) while in women it was probably significant ($p < 0.05$) FEV FEV % MVV_{40} and MVV_{free} were all decreased in both sexes ($p < 0.001$) The ratio FEV_1/FIV_1 was 0.53 in men and 0 66 in women both values were low compared to 0 88 found in normal subjects by Simonsson (30)

Group B *Static lung volumes (l BTPS)* VC and TLC were significantly decreased in both men and women

Tabl I Spirometric data in group B. For symbols, see methods.

Subject no.		Sex	TLC		VC	RV	FRC
				l	% pred.		
1	H.B	M	5.20	93	61	.27	3.47
	E.E.	M	5.9	.2?	40	1.37	.17
3	R.U	M	5.41	3.69	6	1.73	3.61
4	V.S	M	3.60	44	51	1.16	.38
5	J.S	M	3.55	2.7	46	1.28	09
6	B.F	M	5.46	89	55	7	3.93
7	C.Z	M	4.0	3.38	71	1	3.01
8	F.O	M	3.91	.29	42	1.65	79
9	E.P	M	4.31	1.0	33	61	3.18
10	C.M.	M	5.57	3.4	67	15	3.4
11	I.H	M	4.67	1.80	47	.07	3.54
12	V.R.	M	5.01	3.49	7	1.55	.39
13	C.J	F	3.?6	1.90	47	1.34	1.9
14	S.H	F	47	1.83	[illegible]	0.11	1.0?
15	H.B	F	71	0.93	?9	1.81	.09
16	V.D	F	3.8	1.68	47	1.0	.39
1	I.P	F	3.5	1.81	60	1.44	.29
Men		–	4.61	71		1.0	3.0
n 1		SD	0.8	0.15	14.16	0.8	0.61
		Range	3.5–5.57	1.0–3.6	33–	1.16–.87	09–3.93
Women		–	3.0	1.63	48	1.13	1.95
		SD	0.45	0.40	11.95	0.0	0.55
		Range	2.47–3.35	0.93–1.0	?–60	0.11–1.	1.0?–.39

(p <0.001). RV averaged 80 of the predicted value (p <0.05) in men and 101 in women (p >0.05). FRC was on average 3 % of the predicted value in men (p <0.001) and 88 % in women (p >0.05). As the reduction in TCL was greater than the reduction in RV and FRC, the ratios of the two latter to the former were increased.

Dynamic lung volumes (l BTPS). FEV averaged 3 of the predicted value in men and 81 of the predicted value in women. In b th sexes the differences between observed and p d values was non-significant (p >0.05). FEV₁, MVV₄₀ and MVV_free were significantly decreased in both sexes. The ratio FEV₁/FIV₁ averaged in men and women 0.80 compared to 0.88 reported by Simonsson in normal subject (30).

From Tables IV and V it can be seen that in groups A and B some patient had higher MVV than MVV_free. The significantly small dynamic lung volumes in group A and VC and TLC in group B were partly influenced by the criteria of selection.

RV/TLC %	FRC/TLC %	FVC	FEV	%	FEV % % pred.	FEV_1/FIV_1	MVV_{40}	MVV_{free}
44	67	2.58	1.81	67	96	0.65	63	78
38	60	2.31	1.60	69	91	—	49	58
52	67	3.85	2.97	76	103	0.80	100	143
32	66	2.50	1.65	66	96	0.87	48	92
36	59	2.23	1.57	70	96	0.70	56	81
47	72	2.70	1.87	68	97	0.72	71	93
31	62	3.40	2.00	59	88	1.08	41	45
42	71	2.25	1.15	49	67	0.59	37	51
61	74	2.50	1.45	85	116	1.32	37	53
39	62	3.40	.50	69	103	0.76	84	108
62	76	1.75	0.95	54	86	0.59	26	28
31	61	3.14	1.98	57	79	0.68	52	57
42	60	1.54	1.02	55	66	0.56	35	46
26	41	1.76	1.65	86	106	0.98	56	45
66	76	0.95	0.70	64	81	0.88	16	36
53	67	2.10	1.55	74	94	0.94	38	44
44	70	1.40	0.95	54	72	0.63	31	38
41	66	2.72	1.79	66	93	0.80	55	73
10.81	5.78	0.61	0.54	9.83	12.51	0.22	21.24	33.41
31—62	59—76	1.75—3.85	0.95—2.97	49—85	67—116	0.59—1.32	26—100	28—143
46	63	1.55	1.17	67	84	0.80	35	42
14.74	13.48	0.43	0.41	13.52	16.28	0.19	14.38	4.49
26—66	41—76	0.95—2.10	0.70—1.65	54—86	66—106	0.56—0.98	16—56	36—46

Table 17 Comparison between groups A and B spirometric measurements as percentage of predicted normal values.

		TLC % pred.	VC % pred.	RV % pred.	FRC % pred.	FEV % pred.	FEV % % pred.	MVV % pred.
Men	A = 16	99	66	161	122	42	60	38
	B = 12	63	55	80	73	52	93	47
Women	A = 8	100	65	184	134	37	60	30
	B = 5	64	48	104	88	43	84	38
P	Men	<0.001	>0.05	<0.001	<0.001	>0.05	<0.001	>0.05
A—B	Women	<0.001	>0.05	<0.01	<0.01	>0.05	<0.001	>0.05

Total hemoglobin blood volume hemoglobin concentration and heart volume

Total amount of hemoglobin in group A averaged in men 8.92 g/kg body weight SD ± 1.46 and in women 8.18 g/kg body weight SD ± 0.88 In group B the average for men was 9.28 g/kg body weight, SD ± 0.96 and for women 7.72 g/kg body weight, SD ± 0.76 The mean values for men in both groups were significantly lower than normal values, for A $p < 0.001$ and for B $p < 0.01$ In our laboratory (8) normal values are 10.23 ± 1.18 g/kg for men and 8.07 ± 0.85 g/kg for women.

Blood volume in group A averaged in men 72.3 ml/kg body weight, SD ± 12.8 and in women 72.3 ml/kg body weight SD ± 11.9 The values for men were slightly lower than normal values $p < 0.05$ In group B the average for men was 81.0 ml/kg body weight SD ± 15.0 and for women 63.5 ml/kg body weight SD ± 5.4 The males values did not significantly deviate from normal values while the female values were significantly lower $p < 0.01$ Normal values are for men 82.4 ± 8.9 ml/kg and for women 73.9 ± 7.5 ml/kg (8)

Hemoglobin concentration in group A averaged in men 13 [illegible] g/100 ml (range 11 [illegible] 16.6) and in women 12.5 g/100 ml (range 11.2 13.8) In group B it was 12.8 g/100 ml in men (range [illegible] 16.2) and 12.5 g/100 ml (range 10.1 14.1) in women

H [illegible] in group [illegible] in [illegible] A [illegible] averaged [illegible] ml [illegible] [illegible] 1114 and in [illegible] [illegible] 30 In group B [illegible] 841 [illegible] (range [illegible])

626 ml (range 430—770) for women. In group A the individual values varied by 2 SD in relation to THb except in six patients (Nos. 2, 6 8 12, 20 and 21) (Fig 1) In group B the heart volume was normal in relation to THb, except in two patients whose volume was above 2 SD (Nos. 4 and 7) (Fig 2) Both patients had signs of cardiovascular disease However the values in both groups were within ± 2 SD (except in one patient A 12) when compared to the regression line found in elderly normal subjects aged 56—83 (35) In both groups the mean heart volume was between the regression lines of the young and elderly subjects which is normal for the age of the groups. However in the restrictive group 10 of 16 observations fell below the regression line for elderly subjects. HV could not be determined in two patients in group A nor in one patient in group B because of the radiological lung changes.

THb BV and HV mean values were not significantly different in the two groups, except for a smaller BV in men in group A compared to group B ($p < 0.01$) but the difference was not significant when BV was related to body weight ($p > 0.05$) Table III

Comments

According to the functional classification the patients of group A had obstructive ventilatory dysfunction while group B fulfilled the criteria of restrictive ventilatory dysfunction. However the two groups had practically the same ventilatory capacity Table VI Group A had [illegible] decrease in ventilatory capacity [illegible] decrease in group B was [illegible]

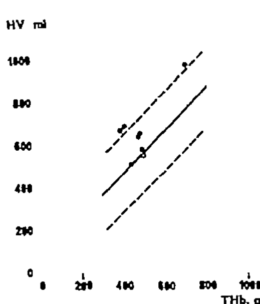


Figure 1 Heart volume (HV) in relation to total amount of hemoglobin (THb) in 16 men (solid symbols) and 6 women (open symbols) with chronic obstructive lung disease. Whole line normal regression line. Interrupted lines ± 2 SD (5 17 21)

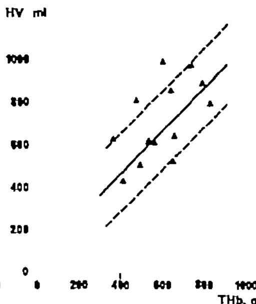


Figure 2 Heart volume (HV) in relation to total amount of hemoglobin (THb) in 11 men (solid symbols) and 5 women (open symbols) with chronic restrictive lung disease. Whole line normal regression line. Interrupted lines ± 2 SD (5, 17 21)

The low ventilatory capacity in the restrictive group is attributed to the reduced vital capacity (see correlation matrix, Chapter VI) Theoretically these patients should be able to counteract the effect of reduced vital capacity by increasing their rate of breathing This is possible if there are no factors limiting the speed of movement. In fact these patients have stiff lungs and thoracic cage (two patients had thoracoplasty) In the obstructive group THb and BV were reduced in men but not in women, probably because men have a higher degree of physical inactivity in relation to their normal life activity In the restrictive group men had a reduced THb but rather normal BV that is to say they were slightly anemic.

The mean heart volume was normal in relation to THb in both groups but showed wide individual variations.

Groups A and B were comparable in many respects, being identical in age, body size and circulatory dimensions, (THb, BV and HV) Table III

References

1 American Thoracic Society. Chronic bron- hiti asthma and pulmonary emphysema. *Amer R p Dis 85 76* 196..

2 Berggren, S.M.: The ox gen deficit of a terial blood caused by the non- ventilating part of the lung. *A ta phy iol s d 4* suppl 11 1942

3 Berglund E., Birath, G. Bj re J. Grimby G. Kjellmer I. Sandqvi t L. & Soder holm, B. Spirometric studies in normal subject. I. Forced expirograms in subject between 7 and 70 y ars of age. *A t m d sc d 173* 185 1 63

4 Bern t in, L., D'Silva, J.L. & Mendel D. The effect of the ra of bre thing on the maximum bre thing c pa ty determined w h new spirometer. *Th a T* 35 195

5 Bevegård S. Holmgren A. & Jonsson, B. The effect of bod position on th irc lation at rest and d ring exerc se w th pe ial referenc to the influenc on the trok ... *A t phy iol scand 49* 7 1 60

6 Ek l nd, L.-G. Determination of bl I ... *Sand J lin Lab ... 17* suppl ... 19 5
Ek l und L.-G. C r latory and respi atory adaptat on d ring prolonged exercise. *Acta ph ... d 0* ... ppl ... 1 67

8 Ek ... d L.-G. Unpublished data.

9 ... H. Volumen inefficiens ... Bemer k ... Lun ... den schädl. Lon Raumes ... 44 191 1 38

10 ... E.I. Macl tosh D.J. & Wright C.W. ... ... and pul- ... normal sub- ... [illegible]

11 ... [illegible]

1 ... [illegible]

13 Hald, A. Statistical theory with engineer ing pplications. John W ley & Sons, New York 1960.

14 Holmgren, A. Determination of the func tional residual olume by means of the he lium dilution method. *Sca d J clin Lab I ... t 6* 131 1954

15 Holmgren, A. & Mattsson H.: A new er gometer with consta t load at arying pedalling rate. *S d J I Lab I vest 6* 137 1954

16. Holmgren A. & Pernow B. Spectrophoto- metric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison with the n Slyke method. *S and J I Lab I st 11* 143 1959

17 Holmgren A. Jonsson, B. Levander M. Linderholm, H. Sj trand T. & Ström, G. Low physical working apacity in sus- pected he rt ses due to inadequat ad justment of peripheral blood flow (so- regulatory ... *A t med sc d 158* 413 1957

18. Holmgren, A.: On the reproducibil ty of steady tate D_{LCO} me ... d ring exerc se in man. *S and J I ... t 17* 110 1965

19 Holmgren, A. E perience of measu ng the diff sing apac ty d ring ... the ady st te met od of ... ed by Linderholm. ... *Pulm ... paci y* ... Ed M. Scherrer H. H ... er Publishers, 1971

20. Ka per ... Limiting factors for aerobic muscle performance. *A t physiol sc d* Suppl 346 19 0

21 Kjellberg S.R. Rudh L. & Sj ... T. The relation of the ... to the weight and ... area of t e bod ... the bl od volume and the ph sical ... ty for work. *A t ... al 31* 113 1949
Larsson H. & Kjellberg S.R. Roentgeno- ... heart vol me determina on w th ... ... of the ... *A ... Rad 29* 159 1948

23. Linderholm, H. & Sjöstrand, T. Determination of carbon monoxide in small gas volumes. *Acta physiol. scand. 37* 240, 1956.

24. Linderholm, H. On the significance of CO-tension in pulmonary capillary blood for determination of pulmonary diffusing capacity with the steady state CO method. *Acta med. scand. 156* 413 1957

25. Linderholm, H., Sjöstrand, T. & Söderström, B. A method for determination of low carbon monoxide concentration in blood. *Acta physiol. scand 66* 1 1966

26. Mellemgaard, K. The alveolar-arterial oxygen difference in chronic obstructive lung disease. *Scand J. resp. Dis. 48* 23 1967

27. Pappenheimer J.R. et al. Standardization of definitions and symbols in respiratory physiology *Fed. Proc 9* 602, 1950.

28. Riley R.L. & Cournand, A. "Ideal" alveolar air and the analysis of ventilation-perfusion relationships in the lungs. *J. appl. Physiol. 1* 825 1949

29. Siggard-Andersen, O.S. Engel, K., Jørgensen, K. & Astrup P. A micro method for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. *Scand J clin. Lab Invest 12* 172, 1960.

30. Simonsson, B.G.. Dynamic spirometry studies in patients with lung disease. *Acta allergol. 18* 386, 1963.

31. Sjöstrand, T. Changes in the respiratory organs of workmen at an ore smelting works. *Act. med. scand* suppl. 196 687 1947

32. Sjöstrand, T. A method for the determination of the total haemoglobin content of the body *Act. physiol scand 16* 211 1948.

33. Sjöstrand, T. *Clinical Physiology* Scand. University Books, Svenska Bokförlaget, Stockholm, 1967

34. Snedecor G.W. *Statistical methods*. Iowa State College Press, Iowa, 1959

35. Strandell, T. Circulatory studies on healthy old men with special reference to the limitation of the maximal physical working capacity *Acta med. scand.* suppl. 414 1964

36. Wahlund, H.. Determination of physical working capacity *Act. med. scand* suppl. 215 1948.

CHAPTER III

EXERCISE TOLERANCE IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

The introduction of exercise tolerance testing in clinical medicine has increased our ability to assess derangements in pulmonary and circulatory functions (11 18) The physical working capacity (PWC) is measured in many ways, depending on the type of patient and the aim of the assessment. In patients with cardiovascular disease PWC can be determined from the heart rate-work load or the heart rate-oxygen uptake relationship, using maximal or submaximal loads, (1 18) In patients with chronic lung disease, in whom working capacity is limited by respiration, PWC can be determined as the maximal performed work of a few minutes duration (10)

The purpose of the present investigation of patients with obstructive and restrictive lung disease was to study 1) the rate of work at the breaking point of exercise (W_B and its relation to respiratory data, 2) the rate of work at heart rate 130 beats/min (W_{130} and W_E, and their relations to circulatory dimensions such as heart and blood volumes 3 the variation of heart rate at the exercise breaking point HR_B) within and between the obstructive and restrictive groups of patients

Results

Orthostatic test. Tables I—II In group A the heart rate (HR) at rest in supine position averaged 84 SD ± 15 and in group B 76 beats/min, SD ± 14 In standing position HR increased significantly in both groups ($p < 0.001$) and in group A averaged 98 beats/min, SD ± 15 and in group B 94 beats/min, SD ± 17 The mean values at rest and the mean heart rate in standing position for each group were within normal variation, but the interindividual variations were large. Five patients in group A (Nos. 3 4 11 15 and 24) and two in group B (Nos. 3 and 16) had a heart rate in supine position of ≥95 beats/min, in standing position ten patients in A and four in B had a marked increase in heart rate to a level >100 beats/min this included the patients with high initial supine heart rate. One case in group A (No. 20) showed a vasovagal reaction on standing, as revealed by a lower HR in standing than in supine position.

Exercise tolerance test Tables I—II In group A the W_B in sitting position averaged in men 432 kpm/min, SD ± 231 range 100—900) and in women 309 SD ± 121 (range 100—400) In

Table I Exercise tolerance test in group A. HR = heart rate, HR_B = breaking point heart rate, W_B = rate of work at the exercise breaking point, W_{130} = rate of work at heart rate of 130 beats/min.

Subject no		REST		EXERCISE						
		HR		HR_B beats/min.		W_B kpm/min.		W_{130}		Cause of interruption in sitting position
		Supine	Standing	Sitting	Supine	Sitting	Supine	Sitting	Supine	
1	U.A	92	109	175	120	565	565	300	500	dyspnea — high heart rat
2	K.E.	46	64	110	121	450	465	—	—	dyspnea
3	H.F	99	108	170	141	600	375	250	250	dyspnea
4	E.E.	102	116	153	154	235	200	—	130	dyspnea
5	W.K.	70	86	137	140	665	600	700	500	dyspnea
6	R.K.	80	86	170	167	900	900	600	600	high heart rate
7	S.J	74	86	152	129	800	535	600	535	dyspnea — fatigue in the legs
8.	W.M.	86	94	118	116	400	265	500	—	dyspnea
9	G.F	80	94	126	120	400	335	450	—	dyspnea
10.	H.M.	72	84	92	—	100	—	—	—	dyspnea
11	E.E.	98	116	138	129	315	285	200	265	dyspnea
12.	A.O	84	100	98	116	250	200	—	—	general fatigue
13	J.L.	88	104	113	108	150	150	—	—	dyspnea
14	A.E.	92	104	147	142	235	200	200	130	dyspnea — ectopic beats
15.	V.W	104	116	134	104	550	400	400	—	dyspnea
16.	P.E.	84	98	132	130	300	265	300	265	dyspnea
17	S.F	82	100	136	162	400	400	250	270	dyspnea — fatigue in the legs
18.	E.K.	84	114	132	110	235	200	—	—	dyspnea — general fatigue
19	E.A.	80	96	134	106	250	65	300	—	dyspnea — fatigue in the legs
20.	B.W	92	88	162	136	400	265	230	230	dyspnea
21	A.F	50	70	132	96	400	400	400	—	dyspnea
22.	E.P	80	102	157	134	450	400	270	370	dyspnea
23.	A.S.	84	94	113	114	100	100	—	—	dyspnea
24.	L.H	116	132	150	142	235	235	—	—	dyspnea

Table II Exercise tolerance test in group B. Symbols as in Table I.

Subject no.		REST HR		EXERCISE HR_M beats/min.		W_D kpm/min.		W_{130}		Cause of interruption in sitting position
		Supine	Standing	Sitting	Supine	Sitting	Supine	Sitting	Supine	
1	H.B.	74	84	148	138	400	265	300	200	dyspnea
2	E.E.	72	90	128	102	400	265	400	—	dyspnea
3	R.U	102	131	167	157	600	535	200	230	dyspnea
4	V.S.	68	80	120	132	400	400	470	400	dyspnea
5	J S	77	82	161	140	700	600	500	500	dyspnea
6	B E	85	102	140	121	465	400	450	470	dyspnea — general fatigue
7	G Z	68	86	128	129	335	265	335	465	dyspnea
8	E O	58	94	131	125	400	400	400	450	ectopic beats
9	E P	68	88	109	141	600	535	600	400	dyspnea — cough — general fatigue
10	G.M.	70	92	109	102	400	200	—	—	ST depression — ectopic beats
11	L.H.	84	96	119	118	250	700	—	—	dyspnea
12	A R.	55	72	160	157	800	700	550	450	dyspnea — fatigue in the legs
13	C.J	66	78	170	150	635	535	400	450	dyspnea
14	S.H.	68	92	138	122	450	400	570	470	dyspnea — fatigue in the legs
15.	H.B.	74	94	144	140	200	200	150	100	dyspnea
16.	A.D.	100	122	140	132	500	350	150	370	dyspnea — cough — general fatigue
17	L.P	94	118	136	133	400	200	200	200	dyspnea

group B the average in men was 479 kpm/min, SD ± 161 (range 250—800) and in women 397 kpm/min, SD ± 164 (range 200—635) The mean values were significantly lower than values predicted from the equation given by Irnell and Linder in normal subjects (8) There was no difference of significance between the group means for either sex ($p > 0.05$) As men and women were included in one group W_B for men and women together in group A was 391 kpm/min, SD ± 207 corresponding to 52 % of the predicted value, and in group B 455 kpm/min, SD ± 162, or 55 % of the predicted value.

In group A the W_B in sitting position was studied as a dependent variable in relation to measurements of lung function, size of the cardiovascular system and body size (Table III) The highest significant correlations were with the indices of the ventilatory capacity FVC, FEV MVV_{40} and MVV_{free} ($p < 0.001$) The second highest correlations were with RV/TLC % VC and W_{130} ($p < 0.01$) The correlation between W_B and MVV_{40} was higher than that with W_{130}, although only values from 16 patients in whom W_{130} could be determined were included in the analysis ($r = 0.845$ $p < 0.001$) There was a probably significant correlation to FEV % FRC/TLC, THb BV and weight ($p < 0.05$) No correlation was found to TLC, RV FRC, HV height or the respiratory rate at work loads 200 and 400 kpm/min (RR_{200} and RR_{400}) Except for RV/TLC % and FRC/TLC % all correlations were positive. The regression of W_B on FEV is shown in Fig 1 When W_B in group B was compared with the same independent variables listed in Table III there was a significant correlation to THb $r = 0.704$ $p < 0.01$ $n = 17$ There was a correlation of probable significance with BV ($r = 0.547$ $n = 17$) weight ($r = 0.601$ $n = 17$) W_{130} ($r = 0.609$ $n = 15$) and RR_{200} ($r = 0.591$ $n = 14$) No significant correlation was found between W_B and VC, nor was there a correlation when only eight patients with VC < 50 % of the predicted value were included. Similarly there was no correlation to any other static or dynamic lung volumes. The relationship between W_B and FEV in group B is illustrated in Fig 2.

In group A the W_{max} in sitting position in men averaged 409 kpm/min, SD ± 174 $n = 11$ and in women 290 kpm/min, SD ± 67 $n = 5$ In group B the figures were for men 441 kpm/min, SD ± 163 $n = 10$ and for women 258 kpm/min, SD ± 118 $n = 5$ The mean values are significantly low compared to normal values (8) In group A the W_{130} was significantly correlated to heart volume and height ($p < 0.01$) There was a correlation of probable significance with THb, BV weight, FVC, FEV and MVV_{40}. In group B the W_{130} was highly correlated to THb ($p < 0.001$) and significantly correlated to BV and weight ($p < 0.01$) The correlation with HV was probably significant ($p < 0.05$) The relationships with other variables were not significant.

HR_B in sitting position averaged 138 beats/min in both groups. Only three patients in group A and two in group B had a heart rate around 170 beats/min. All other patients in both groups inter

Table III Summary of correlation analysis.

Dependent variable	W_B		HR_B		W_{130}	
Independent variable	A	B	A	B	A	B
TLC	—	—	—	—	—	—
VC	++	—	—	—	—	—
RV	—	—	++	—	—	—
FRC	—	—	++	—	—	—
RV/TLC %	++	—	++	—	—	—
FRC/TLC %	++	—	++	—	—	—
FVC	+++	—	+	—	+	—
FEV_1	+++	—	++	—	+	—
FEV %	+	—	++	—	—	—
MVV	+++	—	+	—	+	—
MVV_{free}	+++	—	+	—	—	—
THb	+	++	—	++	+	+++
BV	+	+	—	+	+	++
HV	—	—	—	—	++	+
Height	—	—	—	—	++	—
Weight	+	+	—	+	+	++
W_{130}	++	+	—	—		
RR_{200}	—	+	—	—	—	—
RR_{400}	—	—	—	—	—	—

+ <0.05
++ <0.01
+++ <0.001
— non-significant

rupted the test before reaching a heart rate of 170 beats/min. The cause of interruption of the test (Tables I—II) was mostly dyspnea, in a few cases fatigue in the legs or general fatigue. In group B the cause of interruption was coronary insufficiency and ectopic beats in patient 10 and ectopic beats in patient 8

In group A low HR_B in sitting work was found in patients with high RV FRC, RV/TLC % and FRC/TLC % and low ventilatory capacity HR was not correlated to W_{130}, nor was there any correlation of significance to THb BV HV height, weight, RR_{200} or RR_{400}. In group B the HR_B was correlated to THb ($p < 0.01$) BV and weight ($p < 0.05$) No correlation was found with any of the other variables listed in Table III

The summary of the correlation analysis as regards W_B, HR_B and W_{130} is given in Table III

The mean heart rate at work load 200

Table IV Exercise tolerance test, differences between group means (A minus B) in sitting and supine position, mean values ± SD, number of observations in parentheses.

Variable		Group A	Group B	$\bar{d}$	P
HR, rest-supine		84.0 ± 15.4 (24)	75.5 ± 13.5 (17)	8.5	—
HR, standing		98.3 ± 15.3 (24)	93.8 ± 16.6 (17)	4.5	—
HR_{200}	sitt.	111.5 ± 18.1 (20)	107.3 ± 16.5 (15)	4.2	—
	sup.	112.5 ± 18.0 (18)	111.5 ± 14.3 (16)	1.0	—
RR_{200}	sitt.	24.5 ± 4.5 (20)	24.8 ± 7.0 (14)	— 0.3	—
	sup.	24.8 ± 4.5 (16)	27.2 ± 9.5 (12)	— 2.4	—
HR_{400}	sitt.	131.1 ± 21.6 (12)	125.3 ± 15.9 (12)	5.8	—
	sup.	121.6 ± 21.6 (7)	128.4 ± 7.3 (8)	— 6.8	—
RR_{400}	sitt.	27.3 ± 7.5 (11)	28.6 ± 5.7 (12)	— 1.3	—
	sup.	29.4 ± 7.5 (9)	28.9 ± 6.9 (8)	— 0.5	—
W_{30}	sitt.	371.9 ± 157.2 (16)	379.7 ± 169.9 (15)	— 7.8	—
	sup.	337.1 ± 160.2 (12)	354.6 ± 119.6 (14)	—17.5	—
W_{D}	sitt.	391.0 ± 207.1 (24)	455.0 ± 161.5 (17)	—64	—
	sup.	335.0 ± 183.9 (23)	379.0 ± 157.1 (17)	—44	—
HR_{D}	sitt.	137.5 ± 22.6 (24)	138.1 ± 18.8 (17)	— 0.6	—
	sup.	127.7 ± 18.6 (23)	131.7 ± 16.0 (17)	— 4.0	—

and 400 kpm/min HR_{200}, HR_{400}, (Table IV) was in both groups higher than in normal subjects (5).

The respiratory rate at work load 200 and 400 kpm/min RR_{200}, RR_{400}, (Table IV) was in both groups higher than reported in normal subjects by Grimby and Söderholm (5). The inter

T bl 1 The effect of body position on the measurements during exercise tolerance tests, analysed as difference between the mean values (sitting minus supine)

Variable	Group A					Group B				
	$\overline{d}$	SD_d	C	n	P	$\overline{d}$	SD_d	C	n	P
HR, rest, beats/min. (supine — standing)	—14	6.3	4.9	24	**	—18.4	7 7	6 4	17	***
HR_{300}, beats/min.	— 1.8	15.3	9.6	18	—	— 4 4	11 8	7.6	15	—
RR_{300}, cpm	— 1 4	3 1	9 1	16	—	— 2 1	3 6	9.8	12	—
HR_{400}, beats/min.	4 4	15.6	8.9	7	—	— 5.5	15.3	8.6	8	—
RR_{400}, cpm	— 2.7	4 7	11 7	9	—	— 0.3	3.9	9.5	8	—
W_{130}, kpm/min.	— 1.3	102	20.4	11	—	23 6	138 7	26.8	14	—
W_B, kpm/min.	69	78	14.9	23	***	76	68.2	11.6	17	***
HR_B, beats/min.	12	17.3	9.2	23	**	6 4	13 7	7 1	17	borderline significance

$\overline{d}$ = mean difference· SD_d = standard deviation of the difference C = coefficient of variation n = number of observations P = level of significance

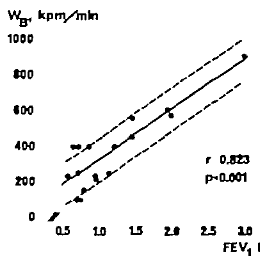


Fig 1 Relationship between W_B and forced expiratory volume in one second (FEV_1) in group A, 24 patients with obstructive lung disease Regression line ± 1 SD Y = 39.2 + 282.3 × SD 120.

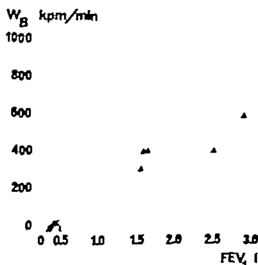


Fig 2 Scatter diagram illustrating the relationship between W_B and FEV_1 in group B, 17 patients with restrictive lung disease

individual variation was also large at 200 kpm/min the range in group A was 17—32 cpm, n = 20 and in group B 16—39 cpm, n = 14 At 400 kpm/min the range in group A was 18—44 cpm, n = 11 and in group B 18—37 cpm, n = 12.

In group A the RR_{200} and RR_{400} were not correlated to VC, FEV_1 or MVV_{40}, while in group B there was a negative correlation of probable significance between RR_{200} and VC (r = —0.652) FEV_1 (r = —0 616) and MVV_{40} (r = —0.547 n = 14) No correlation was found between RR_{400} and those three variables.

The effect of body position

In standing position the mean heart rate was significantly higher than in supine position in both groups ($p < 0\ 01$) W_B was significantly higher in sitting than in supine position in both groups ($p < 0\ 001$) The mean difference was 69 kpm/min in group A and 76 kpm/min in group B The mean HR_B was also higher in sitting position than in supine position, the mean difference in A being 12 beats/min ($p < 0\ 01$) and in B 6 beats/min (borderline significance) There was no difference of significance between mean values in sitting and supine position as regards HR_{200}, HR_{400}, W_{130}, RR_{200} or RR_{400} (Table V)

Comparing the measurements from the exercise tests in both groups, no significant differences between the group means were found (Table IV)

Discussion

According to the functional classification the patients of group A had an obstructive ventilatory impairment, while those of group B fulfilled the criteria of restrictive ventilatory impairment. The two groups were comparable in many respects — age, body size and dimensions of cardiovasculary system — as evidenced by the absence of significant differences between the group means (Chapter II) They had practically the same ventilatory capacity and were also similar in their ability for exercise. The three exercise parameters W_B, W_{130} and HR_B were all decreased in both groups, with no significant differences between A and B

W_B. The maximal work performance in a type of exercise as used in this study is dependent on the oxygen transporting capacity In the *obstructive group* the low W_B was highly related to the low indices of the ventilatory capacity — FVC, FEV_1 MVV_{40} and MVV_{free}. W_B was better correlated to MVV_{40} than MVV_{free} because the first is more reproducible. The higher correlation between W_B and FEV than with FEV % is due to the fact that the latter is probably a qualitative rather than quantitative index of obstruction (16)

The positive relationship between W_B and W_{130} ($p < 0\ 01$) the latter being a relative measure of the stroke volume (20) indirectly signifies an association between W_B and the stroke volume, and consequently the low values of W_B found in these patients should be partly attributed to a small stroke volume. However the correlation between W_B and W_{130} was of a lower magnitude and significance compared to the correlation between W_B and the indices of ventilatory capacity which might indicate that the

stroke volume was a less important limiting factor than the ventilatory capacity Another reason is that W_B represents subjective maximal work, which does not coincide with the maximal work estimated by extrapolation from submaximal heart rate. Bouhuys and Pool (3) showed, in patients with chronic lung disease whose work performance was limited by the ventilatory reserves, that the oxygen uptake at subjective maximal work is lower than the maximal oxygen uptake determined by extrapolation from submaximal heart rate.

The slight correlation between W_B and THb BV and the absence of correlation with HV show that the dimensions of the cardiovascular system are of minor or no importance for the limitation of W_B in these patients. The findings in this study are in agreement with those of Jones *et al* (9) who found that effort tolerance in patients with chronic airway obstruction was largely limited by a low ventilatory capacity Also Irnell (7) found a significant correlation between the maximal performed work and the ventilatory capacity in asthmatic patients. A similar relation was found in patients with chronic bronchitis by Simonsson *et al.* (17) The results differ however from those found in healthy elderly people, in whom no significant correlation was found between maximal performed work and the functional capacity of the lungs or the circulatory dimensions (4 21)

In contrast to the findings in the obstructive group, W_B in the *restrictive group* was not related to any of the static or dynamic lung volumes. However there was a positive relationship between W_B and THb BV weight and W_{130}. Consequently the low W_B in these patients might be ascribed to the reduced circulatory dimensions. But it is rather difficult to attribute the low W_B to the small circulatory dimensions alone especially HR_B was rather far from being maximal. Also the weak correlation with BV and W_{130}, and the absence of correlation with the heart volume, may suggest the coexistence of other limiting factors.

W_{130} The physical working capacity determined as the rate of work at heart rate 170 beats/min (W_{170}) is an estimate of the oxygen pulse and is dependent on the stroke volume and arteriovenous oxygen difference. In patients with severe degree of disability extrapolation to a lower heart rate than 170 has been used, i.e. heart rate 160 150 or as in this study 130 and the rate of work at this heart rate (W_{130}) can be used as relative measure of the stroke volume. In group A W_{130} was related to HV THb BV and body size, which is in agreement with the findings in normal subjects (21) However the relationship with THb and BV was only of probable significance, which can partly be explained by the absence of steady-state conditions in some patients when W_{130} was determined. The slight relationship between W_{130} and FVC, FEV_1 and MVV_{40} suggests the probable importance of these parameters for the filling and emptying conditions of the heart. In group B W_{130}, as in normal subjects, was significantly related to the measurements of the cardiovascular system but was not related to the measurements of lung functions. This shows that the low

W_{130} was not attributable to the direct effect of restrictive ventilatory dysfunction.

HR_B. In normal subjects the physical working capacity is determined mainly by the stroke volume (2 20) In patients with low PWC and having a small stroke volume the cardiac output is secured by increasing the heart rate to maximal level, which is reached at low work loads. In groups A and B the HR_B did not reach maximal levels except in a few cases, which indicates that the cardiovascular system was not stressed to maximal level. In the *obstructive group* HR_B, in the same way as W_B, was related to the measurements of the ventilatory capacity It was, moreover negatively correlated to RV FRC, RV/TLC % and FRC/TLC % thus reflecting the relationship between the high degree of obstruction and the early interruption of exercise. In the *restrictive group* the relationship between HR_B and THb BV and the absence of correlation with HV static and dynamic lung volumes, show the parallelism between HR_B and W_B.

The higher than normal heart rate at submaximal work loads (HR_{300}, HR_{400}) in the two groups signifies a smaller than normal stroke volume at these work loads.

The respiratory rate at submaximal work load was not related to the degree of airway obstruction. A similar finding has been reported by others (17) In the restrictive group a slight negative correlation was found between the vital capacity and the respiratory rate at 200 kpm/min This may be explained by the patients attempt to counteract the effect of reduced vital capacity by increasing their respiratory rate. However the relationship was not significant at higher level of ventilation (400 kpm/min) probably due to the involvement of other factors affecting the work of breathing which force the patients to choose their own optimal frequency of breathing (6 13) The latter mechanism might explain the large variation in respiratory rates in the two groups.

The effect of body position The lower W_B and HR_B in supine position than in sitting position accord with the findings in elderly healthy men by Strandell (21) who attributed the difference to the effect of hydrostatic counterpressure and to untrained, or a smaller engagement of muscle groups in supine exercise. Other contributory factors may be of importance, e.g the reduction in lung volumes in recumbent position (12 22) irrespective of its mechanism (19 23) causes a decrease in the cross-sectional area of the airways. Consequently the work of breathing becomes higher in supine position because of an increased airway resistance (14 15) These factors explain the lower work performance in supine than in sitting position.

The chang in body position did not have a significant effect on W_{130} and heart rates at submaximal work loads, indicating a minimal orthostatic effect during exercise. Similarly there was no effect on the respiratory rates.

Summary

1 W_B, W_{130} and HR_B were reduced in both the obstructive (A) and restrictive (B) groups.

2 The cause of interruption of the exercise test was dyspnea in 96 % of the patients in group A and 88 % of the patients in group B

3 W_B in group A was highly correlated to the ventilatory capacity ($p < 0.001$) and negatively correlated to RV/TLC % and FRC/TLC % ($p < 0.01$) It was weakly related to the circulatory dimensions. W_B in group B was not related either to the lung volumes or to ventilatory capacity but significantly correlated to THb ($p < 0.01$) and BV ($p < 0.05$)

4 In group A low HR_B was found in patients with low ventilatory capacity and large lung volumes, while in group B it was associated with reduced THb and BV

5 W_{130} in group A was correlated, though weakly with the ventilatory capacity and circulatory dimensions, while in group B it was significantly correlated with the circulatory dimensions.

6 W_B and HR_B were significantly higher in sitting than in supine position in both groups, while W_{130} as well as the heart and respiratory rates at submaximal work were not significantly different in the two positions.

7 The respiratory rate at submaximal work showed wide variations in the two groups. In group A it was not related to the degree of obstruction while in group B RR_{30} was negatively but weakly correlated with the vital capacit

References

1 Astrand, P.O & Rhyming I. A nomogram fo calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. *J ppl Physiol. 7* 218 1954

2. Bevegård, S Holmgren A. & Jonsson, B. The effect of body position on the circulation t rest and during exercise with special reference to the influence on the strok volume. *Acta physiol. scand. 49* 279 1960.

3 Bouhuys, A. & Pool J: Physical working capacity in pulmonary disease (Abstr) *Amer rev R p Dis 88* 103 1963

4 Erleson, P & Irnell, L. Physical work capacity and static lung volumes in elderly people *Acta med scand 185* 185 1969

5 Grimby G & Söderholm, B. Spirometric studies in normal subjects. III, Static lung olumes and maximum voluntary ventilation in adults with a not on physical fit ness. *A ta med scand. 173* 199 1963

6. Hedstrand, U The optimal frequency of breathing in bronchial asthma. *S and. J re p Dis 52* 217 1971

7 Irnell, L. A study of bronchial asthma. *Acta med. scand* suppl. 419 1964

8 Irnell, L. & Linder U published observations. Quoted in ref. 7 p 27

9 Jones N.L., Jones G & Edwards R H.T Exercise tolerance in bronic airway obstruction. *Amer rev R p Dis. 103* 477 1971

10 Linderholm, H. Diffusing capacity of the lungs as limiting factor for physical working capacity *Acta med scand 163* 61 1959

11 Linderholm, H. *F tional pacity d xercise toleranc in patients with impair ed lu g function Clinical ardiopulmonary physiol gy* Grune & Stratton Inc., New York, 1960.

12. Moreno, F & Lyons, H.A. Effect of body posture on lung olumes. *J ppl. Physiol. 16* 27 1961

13 Otis, A.B. The work of breathing. *H nd book of Physiology* section 3 vol. 1 p. 463 American Physiological Society Washington, 1964

14 Petit, J.M., Milic Emili G & Sadoul, P:

L'influence de la position corporelle sur le travail entilatoire dynamique pendent l'exercice musculaire chez l'individu sain. *Arch. internat physiol. Bioch. 68* 437 1960.

15 Rodman, T & Sterling, F.H. *Pulmonary emphysema and elated lu g diseases.* V.C. Mosby Comp., p. 167 1969

16. Simonsson, B G Dynamic spirometry and static lung olume measurements. *Acta allergol. 18* 375 1963

17 Simonsson, B.G Malmborg, R. & Berglund, E. Pulmonary gas exchange at rest and during exercise in chronic bronchitis. *S and J resp Dis. 50* 245 1969

18. Sjöstrand, T.. Changes in the respiratory organs of workmen at an ore smelting works. *Acta med scand.* suppl. 196 687 1947

19 Sjöstrand, T Determination of changes in the intrathoracic blood olume in man. *Act physiol. scand. 22* 114 1951

20 Sjöstrand, T *Clinical Physiology* Scand. University Books, Svenska Bokförlaget, Stockholm, 1967

21 Strandell, T : Circulatory studies on healthy old men with special reference to the limitation of the maximal physical working capacity *A ta med scand suppl.* 414 1964

22. Svanberg, L. Influence of posture on the lung volumes, ventilation and circulation of normals. *S and. J clin Lab Invest* suppl. 25 1957

23 Wade, O.L. & Gibson J C. The effect of posture on diaphragmatic movement and ital capacity in normal subjects, with note on spirometry as an aid in determining radiological chest olumes. *Thorax 6* 103 1951

CHAPTER IV

THE CAUSES OF HYPOXEMIA IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

Arterial hypoxemia is a common feature in chronic obstructive and restrictive lung disease. Patients with chronic interstitial fibrosis and similar clinical conditions often exhibit severe arterial hypoxemia during exercise despite a high alveolar ventilation (4 5) Some investigators (4 30 34) have studied the cause of hypoxemia during exercise in patients with pulmonary fibrosis, but its mechanism has not been fully elucidated The present study was undertaken in order to evaluate the factors contributing to hypoxemia, particularly during exercise, in patients with obstructive and restrictive lung disease.

Procedure

The examinations were carried out early in the morning after a light breakfast. No smoking was allowed after 7 o clock in the evening before examination. A catheter was introduced usually in the left brachial artery by a percutaneous Seldinger technique this was followed by determination of ventilation and alveolar gas exchange at rest in sitting position. After a resting period of about 15 minutes the diffusing capacity was determined during steady-state exercise using 0 05 % carbon monoxide in air The expired air was collected for 3 minutes in a Douglas bag In the middle of this period two arterial blood samples were withdrawn for determination of gas tensions, pH and carbon monoxide content. The work period was as a rule 7 minutes. After a resting period of 30 minutes the nitrogen wash-out time and the veno-arterial shunt were measured. Blood samples for determination of Pa_{O_2} and Pa_{CO_2} were withdrawn at 10 minutes and at the end of the breathing period and analysed immediately within half a minute. The sample with the highest Pa_{O_2} was used in the calculation.

Results

Respiratory data are shown in Tables I—II

In group B no significant difference was found between patients with fibrotic restriction and patients with pleural restriction, either at rest or during exercise, as regards the following variables V_D/V_T $P_{A_{O_2}}$ Pa_{O_2} $P(A—a)_{O_2}$ and $D_{L_{CO}}$ Therefore they were treated as one group.

The oxygen uptake ($\dot{V}_{O_2}$) at rest and during exercise was similar in both groups, $p > 0.05$

Total ventilation (V_E) at rest was

Table I Respiratory data t rest and during ting exercise i group A. 24 patients with chronic obstructive lung disease.

	W kpm/min		$\dot{V}_{O_2}$ ml/min	$\dot{V}_E$ l/min	$\dot{V}_E/\dot{V}_{O_2}$	V_T ml	Resp. rat cpm	V_D ml	V_D/V_T
Rest		$\bar{x}$	287	11 4	40.6	609	20	289	0.48
		range	203—480	8 5 14.9	25.1—56 0	281 —863	12—32	81—146	0.30—0.60
Exercise	245	$\bar{x}$	839	23 0	31 2	1035	26	375	0.39
	100—600	range	406—1464	14.2 —11 4	19 1—46.4	477—1803	15—40	186—686	0 19—0 61

		$\dot{V}_A$ l/min	$P_{A_{O_2}}$ mm Hg	$P_{a_{O_2}}$ mm Hg	$P_{a_{CO_2}}$ mm Hg	pH units	R	$P_{(A-a)O_2}$ mm Hg	$D_{L_{CO}}$ ml/min/ mm Hg
Rest	$\bar{x}$	5.2	102	67	40	7 41	0.82	35	
	range	3.7—7.3	86—115	48—77	30—57	7.35—7.51	0 64—0.95	0—54	
Exercise	$\bar{x}$	14 6	99	64	44	7.35	0.85	35	17.3
	range	5 4—29 1	76—111	39—89	33—71	7.28—7.50	0 70—0.97	20—58	7.0—33.2

= 21

Table II Respiratory data at rest and during sitting exercise in group B. 17 patients with chronic restrictive lung disease

	W kpm/min		$\dot{V}_{O_2}$ ml/min	$\dot{V}_E$ l/min	$\dot{V}_E/\dot{V}_{O_2}$	V_T ml	Resp. rate cpm	V_D ml	V_D/V_T
Rest		$\bar{x}$	290	11.0	38 4	568	20	27	0.41
		range	209—414	7 6—14.9	26.7—54 0	355—872	10— 8	117—363	0.20—0.57
Exercise	84	$\bar{x}$	988	29 6	30 7	1173	27	370	0.32
	100—500	range	610—1471	17 7—43.7	18.1—38.8	587—1722	17—39	136—634	0.11—0.50

		$\dot{V}_A$ l/min	$P_{A_{O_2}}$ mm Hg	$P_{a_{O_2}}$ mm Hg	$P_{a_{CO_2}}$ mm Hg	pH units	R	$P_{(A-a)_{O_2}}$ mm Hg	$D_{L_{CO}}$ ml/min/mm Hg
Rest	$\bar{x}$	5 7	106	74	36	7 43	0.80	32	
	range	4.0—8.8	96—117	56—90	7—44	7.36—7.50	0 72—0.88	18—48	
Exercise	$\bar{x}$	18 6	105	67	40	7.38	0.86	38	17 1
	range	9 6—26...	85—118	5 —88	29—53	7.32—7 43	0.76—0.96	3—50	8 6—29.8

n = 16

high in relation to oxygen uptake in both groups and increased during exercise due to an increase both in respiratory rate and in tidal volume. No significant difference was found between the group means, $p > 0.05$

Alveolar ventilation (V_A) at rest was of the same order in both groups. During exercise it was slightly higher in group B, $p < 0.05$ The alveolar ventilation increased with increasing V_{O_2} in group A according to the equation $V_A = -1.33 + 0.019\ V_{O_2}$ (r = 0.917 SD ± 2.68, n = 24 $p < 0.001$) and in group B according to the equation $V_A = 4.97 + 0.014\ V_{O_2}$ (r = 0.782, SD ± 3.21 n = 16 $p < 0.001$) Thus at a given oxygen uptake V_A during exercise was higher in group B than in group A.

The arterial carbon dioxide tension (Pa_{CO_2}) averaged in group A at rest 40 mm Hg and during exercise 44 mm Hg Considering the normal Pa_{CO_2} range to be between 35—45 mm Hg, 4 patients had moderate to severe elevation of Pa_{CO_2} at rest and during exercise and 3 a slight elevation only during exercise. In group B Pa_{CO_2} averaged at rest 36 mm Hg and during exercise 40 mm Hg One patient had a moderate elevation of Pa_{CO_2} during exercise. In both groups a few patients showed a lower level of Pa_{CO_2} During exercise Pa_{CO_2} increased 4 mm Hg in both groups.

The physiological dead space (V_D) and the *dead space ventilation ratio* (V_D/V_T) were higher at rest than values reported in healthy subjects (20 24 32) V_D/V_T averaged 0.48 in group A and 0.41 in group B. During exercise V_D increased in both groups due to an increase in V_T and respiratory rate. However the increase in V_T exceeded the increase in V_D, so that the ratio V_D/V_T decreased 9 % in both groups, the mean value in group A being 0.39 and in group B 0.32. The difference between the two groups at rest and during exercise was of probable significance $p < 0.05$

Alveolar oxygen tension ($P_{A_{O_2}}$) in group A averaged at rest 102 mm Hg and decreased slightly during exercise. In group B the average was 106 mm Hg at rest and was essentially unchanged during exercise.

The arterial oxygen tension (Pa_{O_2}) was slightly lower in group A than in group B, $p < 0.05$ and averaged 67 mm Hg range 48—77 in group A and 74 mm Hg range 56—90 in group B. The lowest values predicted (mean —2 SD) according to the equation given by Mellemgaard (20) taking into account the decrease of Pa_{O_2} with age, was 76 mm Hg in group A and 77 mm Hg in group B. During exercise the decrease in Pa_{O_2} in group A (with a higher V_D/V_T ratio) was not significant ($p > 0.05$) and was of lower magnitude, 3 mm Hg ($p > 0.05$) than in group B (with a lower V_D/V_T) which showed a fall of Pa_{O_2} 7 mm Hg ($p < 0.05$)

The alveolar arterial oxygen tension difference $P(A—a)_{O_2}$ was at rest large in both groups compared to normal values (20 24 32) In group A it was 35 mm Hg range 0—54 and was essentially unchanged during exercise. In group B the average at rest was 32 mm Hg, range 18—48 and during exercise 38 mm Hg range 23—50 an increase of

6 mm Hg ($p < 0.05$) 11/24 patients in group A and 5/16 patients in group B showed a decrease or an unchanged $P(A-a)_{O_2}$ difference during exercise.

Diffusing capacity of the lungs ($D_{L_{CO}}$) was determined during sitting exercise in 22 patients in group A, of whom one was discarded, and in 16 patients in group B. Two patients in group A and one in group B had to interrupt the work shortly after the start. In group A the mean work load was 245 kpm/min, range 100—600 corresponding to 63 % of the rate of work at the breaking point of exercise (W_B) obtained during the exercise tolerance test. The mean heart rate was 121 beats/min, range 98—155 corresponding to 88 % of the heart rate at the breaking point of exercise (HR_B). In group B the mean work load was 284 kpm/min, range 100—500 corresponding to 62 % of W_B, and the mean heart rate 123 beats/min, range 110—148, corresponding to 89 % of HR_B. $D_{L_{CO}}$ was identical in the two groups and in group A averaged 17.3 ml/min/mm Hg, range 7.0—33.2, and in group B 17.1 ml/min/mm Hg, range 8.6—29.8.

The individual $D_{L_{CO}}$ values in each group were compared to normal values predicted from the equation given by Donevan *et al.* (10) using the age and the oxygen uptake as independent variables. In group A nine patients had $D_{L_{CO}}$ lower than the predicted values, nine had higher values, while three had values equal to predicted values (Fig. 1). In group B nine patients had a reduced $D_{L_{CO}}$, four had higher values and three had values equal to predicted values (Fig. 2). In this material some patients were above 50 years of age, which was given as the highest age in Donevan's material. When only patients aged 50 years or below were included, the mean difference between observed and predicted values was —5.2 units in group A ($p > 0.05$) $n = 6$) and —6.3 units in group B ($p < 0.05$ $n = 9$). The change of $D_{L_{CO}}$ with V_{O_2} in group A was in accordance with the equation $D_{L_{CO}} = 1\,600 + 0.022\ V_{O_2}$ and in group B with the equation $D_{L_{CO}} = -2\,950 + 0.020\ V_{O_2}$. Neither the slope nor the intercept were significantly different in the two groups ($p > 0.05$).

In group A there was a significant correlation between $D_{L_{CO}}$ and both MVV_{40} and FEV_1 ($p < 0.01$) and a probably significant correlation with THb and BV ($p < 0.05$) but no significant correlation with TLC, VC, RV and FRC. In group B the $D_{L_{CO}}$ was significantly correlated to THb ($r = 0.686$, $p < 0.01$) and BSA ($r = 0.659$ $p < 0.01$). There was no significant correlation to the spirometric data. Nor was there a correlation between $D_{L_{CO}}$ and VC in patients with fibrotic restriction or pleural restriction.

The veno-arterial shunt in group A averaged at rest 10.7 % of the cardiac output, range 6.5—18 and in group B 8.7 % range 4.5—13 the difference between the group means not being significant. The mean values were higher than those found in healthy subjects (33).

A further analysis of the results was made by selecting some patients from group B with the greatest fall of Pa_{O_2}

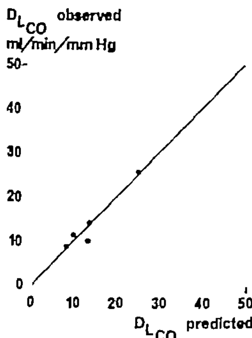


Fig 1 $D_{L_{CO}}$ during exercise observed in 14 men (solid symbols) and 7 women (open symbols) with chronic obstructive lung disease in relation to predicted values. Solid line = identity line.

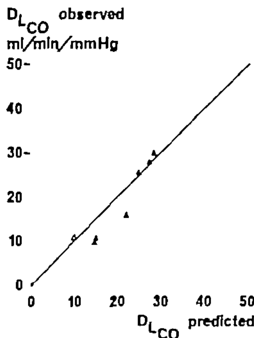


Fig 2 $D_{L_{CO}}$ during exercise observed in 11 men (solid symbols) and 5 women (open symbols) with chronic restrictive lung disease. Solid line = identity line.

during exercise. Six patients were selected (Table III) four of whom had fibrotic restriction (Nos. 1 2, 5 and 6) and two pleural restriction (Nos. 3 and 4) The fall of Pa_{O_2} during exercise in these patients varied between 14—25 mm Hg and the corresponding increase in $P(A—a)_O$ was 7—25 mm Hg. During exercise 1/6 had a normal V_D/V_T ratio and 4/6 had $P_{A_{O_2}} \geq 103$ mm Hg $D_{L_{CO}}$ was normal in relation to age in two patients, moderately reduced in another two, and severely impaired in the remaining two patients. In these patients the overall $V_A/\dot{Q}$ ratio and the diffusing capacity/perfusion ratio, $D_{L_{CO}}/Q$, for the whole lung were calculated for each patient during exercise. As the flow ($\dot{Q}$) was not measured during this examination, $\dot{Q}$-values were calculated using the regression equation for cardiac output on oxygen uptake obtained during hemodynamic study of the patients in this material. It is necessary to point out that the calculated Q-values correspond to values measured in the supine position, while the oxygen uptake in this study was measured in sitting position, which would introduce an error However these values are used solely for comparative purposes. The magnitude of the fall in the arterial oxygen tension during

T bl III Ventilatory data in 6 selected patients with restrictiv lung disease at rest and during exercise.

		V_D/V_T	$P_{A_{O_2}}$ mm Hg	$P_{a_{O_2}}$ mm Hg	$P_{a_{CO_2}}$ mm Hg	$P_{(A-a)_{O_2}}$ mm Hg	$D_{L_{CO}}$ ml	$V_A/\dot{Q}$	$D_{L_{CO}}/\dot{Q}$	$\Delta P_{a_{O_2}}$ mm Hg
1	S.H.	0.42	117	90	24	27				
		0.38	107	65	38	42	12.2	1 68	1.02	25
2.	V.S.	0.35	100	77	42	23				
		0.50	103	55	47	48	9.9	1 47	0.97	22
3.	C.J	0 42	102	84	41	18				
		0 77	106	64	44	42	16 0	1 65	1.24	20
4	E.O	0.44	105	77	37	28				
		0.39	96	61	46	35	16 0	1.30	1.59	16
5.	E.P	0.35	99	79	44	70				
		0 1	93	64	47	29	28.1	1 48	1.85	15
6.	J.S.	0.43	107	75	36	32				
		0 30	110	61	40	49	29.8	1.85	2.19	14

T bl II Comparison between group A and group B entilatory data including calculated $V_A/\dot{Q}$ and $D_{L_{CO}}/\dot{Q}$ at rest (R) and during exercise (E)

		V_D/V_T	$P_{A_{O_2}}$	$P_{a_{O_2}}$	$P_{(A-a)_{O_2}}$	$\Delta P_{a_{O_2}}$	$\Delta P_{(A-a)_{O_2}}$	$\dot{V}_A/\dot{Q}$	$D_{L_{CO}}/\dot{Q}$
A	R	0 48	102	67	35			0 6	—
	E	0 39	99	64	35	—3	0	1.5	1.8
B	R	0 41	106	74	32			0.7	—
	E	0.52	105		38	—7	6	1 7	1.5

alues obta ned during heart ther iza ion

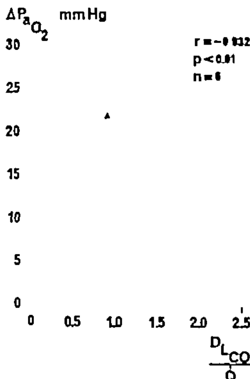


Fig 3 Difference between P_{O_2} at rest and during *exercise* (ΔPa_{O_2}) *in relation to the diffusing capacity/perfusion ratio in six* selected patients with restrictive lung disease

exercise ($\triangle Pa_{O_2}$) showed a high negative correlation with $D_{L_{CO}}/Q$, r = —0.932 p <0 01 (Fig 3) but no significant correlation was found with $\dot{V}_A/\dot{Q}$ or V_D/V_T p >0.05 The mean V_A/Q and $D_{L_{CO}}/Q$ during exercise were then calculated in the same way in groups A and B The V_A/Q ratio was found to be lower in the obstructive group and averaged 1.5 compared to 1 7 in the restrictive group and conversely the $D_{L_{CO}}/Q$ ratio was higher in the obstructive group (1 8) than in the restrictive group 1.5 (Table IV)

Discussion

The point of departure for this discussion is the observation of a significant fall in arterial oxygen tension and increase in $P(A—a)_O$ difference during exercise in the restrictive group despite a high level of alveolar ventilation, in contrast to a minor fall in Pa_{O_2} and unchanged $P(A—a)_{O_2}$ in the presence of low ventilation in the obstructive group

At rest maldistribution of ventilation in relation to perfusion was evidenced in both groups by high V_D/V_T and V_D. This finding is in agreement with previous studies in obstructive lung disease (1 8, 16 21) in pulmonary fibrosis (1 12 25 30 34) also in patients with pleural restriction (18) and with pulmonary tuberculosis treated with pneumothorax (2) The veno-arterial shunt was increased in both groups. If one assumes that the contribution of the diffusion component to the $P(A—a)_O$ gradient at rest is negligible (26) then the low level of Pa_{O_2} and large $P(A—a)_{O_2}$ difference in groups A and B are mainly attributable to inequality of ventilation-perfusion relationship and to increased veno-arterial shunt. These changes were more pronounced in the obstructive group.

During exercise there was an improvement in the distribution of the ventilation in relation to perfusion in the two groups, as evidenced by the lower V_D/V_T However the derangements in the restrictive group were less marked than in the obstructive group, as shown by V_D/V_T and the high level of alveolar ventilation, as also by the absolute value and in relation to the oxygen uptake.

Nonetheless the fall in the arterial oxygen tension was more pronounced in the restrictive group Most studies on the mechanism responsible for hypoxemia in restrictive lung disease have been carried out in patients with pulmonary fibrosis (alveolar capillary block) and been based on the analysis of three classical factors contributing to P $(A—a)_{O_2}$ difference, namely 1) inequality of ventilation-perfusion, 2) venous admixture, 3) diffusion limitation. It has been suggested (34) that hypoxemia during exercise is caused to a large extent by ventilation-perfusion inequalities. Such a change might be caused by a low compliance of the fibrotic alveoli (35) which also leads to uneven ventilation (11) This explanation is based on the increase of ventilation-perfusion inequalities during exercise in patients with fibrotic lungs. In group B however the mean V_D/V_T was significantly lower both at rest and exercise, than the corresponding values in the obstructive group Staněk *et al.* (30) suggested that hypoxemia during exercise in pulmonary fibrosis is caused by the combined effect of a lowering of the oxygen saturation of the venous blood and hypoventilation of some areas. However the hemodynamic study of these patients (Chapter V) showed that, at a particular level of oxygen uptake, the mixed venous oxygen saturation was higher in the restrictive than in the obstructive group Also the ventilatory data (Tables I—II) showed that hypoventilation was prevalent in patients with obstructive lung disease.

It has been suggested (11) that in patients with alveolar capillary block there is an increase of the veno-arterial shunt at rest. This is caused by complete obliteration of the alveoli by the fibrotic process or by the shunting effect of severe impairment of diffusion (29) The behaviour of this shunt during exercise is not clear There is a probability that true shunting contributes to hypoxemia during exercise in alveolar capillary block syndrome (34) However Holmgren and Svanborg (13) showed that in patients with sarcoidosis the shunt is practically constant or decreases during exercise. During oxygen breathing at rest the shunt was lower in the restrictive group than in the obstructive group, but whether this relationship remains unchanged during exercise is difficult to determine.

Impairment of diffusing capacity has been demonstrated in chronic obstructive lung disease (9 17) in pulmonary fibrosis (4 6 9 15) and in pleural restriction (19) It has been suggested that hypoxemia in pulmonary fibrosis is caused by impairment of diffusing capacity due to thickening of alveolar membrane (4 6 9 15) or to its shunting effect (29) Impairment of diffusing capacity may be caused by an inability to expand the pulmonary vascular bed (7) in pleural restriction. The mean $D_{L_{CO}}$ was identical in groups A and B which were also similar in their anthropometric measurements (Chapter II) In the restrictive group the oxygen uptake as well as the alveolar ventilation, tidal volume and respiratory rate were slightly higher than in the obstructive group, and the mean age was slightly lower factors which should have contributed to a higher $D_{L_{CO}}$ in group B (3 10 27) It emerges that the diffus-

ing capacity at a given oxygen uptake was actually lower in the restrictive than in the obstructive group. This is also evidenced by the regression equation of $D_{L_{CO}}$ on V_{O_2}. A decrease in $D_{L_{CO}}$ causes an increase in the alveolar capillary oxygen tension difference. The latter averaged 42 mm Hg (range 26—71) in group A and 47 mm Hg (range 31—71) in group B. These values were far lower than those reported at maximal oxygen uptake (14).

The data for the six selected patients (Table III) showed that the lowering of Pa_{O_2} during exercise cannot be totally attributed to the decreased diffusing capacity per se for example patients E.P and J.S. had normal $D_{L_{CO}}$ values, nonetheless the changes of Pa_{O_2} and P $(A—a)_{O_2}$ during exercise were remarkable. From the data in Table III the following observations could be made 1) Severe lowering of Pa_{O_2} during exercise occurred both in fibrosis and pleural restrictive conditions. 2) It was not always associated with high V_D/V_T but occurred in the presence of *normal* V_D/V_T 3) It is not dependent on the level of the alveolar capillary oxygen tension difference per se.

Although some investigators have found that impairment of $D_{L_{CO}}$ in pulmonary fibrosis was caused by thickening of alveolar membrane (6 9) yet Roughton and Forster (28) have shown that the resistance of the alveolar membrane is only half of the total resistance and concluded that the resistance of the capillary blood to diffusion is of equal importance. Also Staub (31) found that most of the diffusing resistance lies in the capillary blood and not in the alveolar membrane. In this material the low $D_{L_{CO}}$ in the restrictive group was significantly correlated with THb but no correlation was found with the spirometric measurements, which might indicate that the low $D_{L_{CO}}$ was related to derangements on the vascular side and not to ventilatory dysfunction. Staub (31) found that the regional variations in the capillary transit time have a more marked effect on lowering of the oxygen tension than has the membrane variation.

A decrease in capillary transit time causes a decrease in diffusing capacity/perfusion ratio $(D_{L_{CO}}/Q)$ Piiper (22) in his theoretical analysis, showed that the P $(A—a)_{O_2}$ difference is dependent on the regional $D_{L_{CO}}/Q$ ratio rather than on the overall diffusing capacity as such. From the relationship between the oxygen uptake and diffusing capacity $D_{L_{CO}}$, on the one hand, and the oxygen uptake and flow $\dot{Q}$ (Fick principle) on the other the relationship between the diffusing capacity and perfusion is expressed by the following equation

$$\frac{D_{L_{O_2}}}{\dot{Q}} = \frac{C_{a_{O_2}} - C\bar{v}_{O_2}}{P_{A_{O_2}} - P\bar{c}_{O_2}}$$

Thus at a given $P_{A_{O_2}}$ and a given mixed venous blood the oxygen content and partial pressure in the pulmonary capillary and consequently in the arterial blood, are determined by the ratio $D_{L_{O_2}}/Q$. In the six selected patients (Table III) the fall in arterial oxygen tension during exercise (ΔPa_{O_2}) was closely related to the overall $\dot{D}_{L_{CO}}/\dot{Q}$

values, $p < 0.01$ (Fig 3) but no significant correlation was found with the corresponding values of $\dot{V}_A/\dot{Q}$ The mean V_A/Q and $D_{L_{CO}}/Q$ ratios in the two groups showed a contrasting result, namely a low V_A/Q and a high $D_{L_{CO}}/Q$ in the obstructive group while a high V_A/Q and a low $D_{L_{CO}}/Q$ were found in the restrictive group (Table IV)

Read and Williams (25) suggested that ventilation/perfusion inequality in the presence of diffusion defect, contributes to hypoxemia during exercise in patients with pulmonary fibrosis. However the implication of such a mechanism in this study should cause a more marked Pa_{O_2} fall in the obstructive than in the restrictive group. It is apparent, therefore, that the discrepancy in the variation of Pa_{O_2} and $P\ (A—a)_{O_2}$ between the two groups could be attributed to the low V_A/Q in the obstructive group and low $D_{L_{CO}}/Q$ in the restrictive group It seems likely that the contribution of low $D_{L_{CO}}/Q$ to the magnitude of the fall in oxygen tension ($\triangle Pa_{O_2}$) and to the increase in $P\ (A—a)_O$ is more marked than the effect of the variation in V_A/Q when the diffusion defect is identical. Thus the change in level of Pa_{O_2} and $P\ (A—a)_{O_2}$ seemed to depend on the interrelation between V_A/Q and $D_{L_{CO}}/\dot{Q}$, i.e. on which of them predominated

In conclusion, it may be suggested that the changes in arterial oxygen tension and the $P\ (A—a)_{O_2}$ difference during exercise in the obstructive and restrictive groups are explained on the basis of uneven ventilation/perfusion and diffusion/perfusion inequalities and not on the basis of the three classical factors of ventilation/perfusion, diffusion limitation alone or venous admixture, which is in agreement with the concept of Piiper (23)

Summary

1 In the obstructive group (A) the average Pa_{CO_2} was normal at rest and somewhat elevated during exercise Pa_{O_2} was slightly lower than normal but did not fall significantly during exercise in most of the patients.

2 In the restrictive group (B) Pa_{CO_2} was somewhat lower than in group A, both at rest and during exercise, and arterial P_{O_2} was somewhat higher at rest but fell markedly in some patients during exercise.

3 $P\ (A—a)_{O_2}$ was increased in both groups at rest, while during exercise it remained unchanged in group A but increased in group B.

4 V_D, V_D/V_T and Qsh/Q showed high values in both groups, higher in A than in B

5 $D_{L_{CO}}$ was slightly to severely reduced in 9/21 patients in group A and 9/16 patients in group B It averaged 17.3 ml/min/mm Hg in group A and 17 1 ml/min/mm Hg in group B

6 During exercise)
was lower in
the restricti
verse
lated

7 On the basis of these findings it was concluded that hypoxemia during exercise was due mainly to uneven ventilation/perfusion in the obstructive group and uneven diffusion/perfusion in the restrictive group.

References

1 Alnes, E.G Determinations of physiological dead-space and alveolo-arterial oxygen gradients by the indirect methods of Riley and Enghoff. *Scand J lin. Lab Invest. 14* 495, 1962.

2. Alnes, E.G Determinations of physiological dead-space and alveolo-arterial oxygen gradients by the indirect methods of Riley and Enghoff. *Scand J clin Lab Inv st. 14* 507 1962.

3 Anderson, T.W & Shepard, R.J The effects of hyperventilation and exercise upon the pulmonary diffusing capacity *Respiration 25* 465 1968.

4. Austrian, R., McClement, J.H., Renzetti, A.D Donald, K.W Riley R.L. & Cournand, A. Clinical and physiological features of some types of pulmonary diseases with impairment of alveolar-capillary diffusion. The syndrome of alveolar capillary block" *Amer J Med 11* 667 1951

5. Bates, D.V & Christie, R.V *R piratory function in disease* W.B. Saunders. Philadelphia & London, 1964 p. 286.

6. Bates, D.V Varvis, C.J Donevan, R.E. & Christie, R.V Variations in the pulmonary capillary blood volume and membrane diffusion component in health and disease. *J lin. Invest 39* 1401 1960.

7 Bedell, G.N & Adams, R.W Pulmonary diffusing capacity during rest and exercise. A study of normal persons and persons with atrial septal defect, pregnancy and pulmonary disease. *J clin Invest. 41* 1908, 1962.

8 Birath, G Airway and physiologic dead space in patients with obstructive emphysema. *M d Thorac 19* 4 1962

9 Daum, S. Oxygen transferring factor (diffusing capacity) and its components during work in patients with chronic obstructive lung disease and in patients with diffuse interstitial lung disease. *Re piration 28* 479 1971

10 Donevan, R.E., Palmer W.H., Varvis, C.J & Bates, D.V Influence of age on pulmonary diffusing capacity *J ppl. Physiol 14* 483 1959

11 Finley T.N Swenson, E.W & Comroe, J.H. The cause of arterial hypoxemia t rest in patients with alveolar-capillary block syndrome *J clin. I est 41* 618, 1962

12 Holland, R.A.B. Physiologic dead space in the Hamman-Rich syndrome. *Amer J M d 28* 61 1960.

13. Holmgren, A. & Svanborg V Venous admixture during exercise in sitting position. *Scand J clin Lab I vest 17* 209 1965.

14 Holmgren, A. Experience of measuring the diffusing capacity during exercise with the steady state method of Filley modified by Linderholm. I *Pulm ary diffusing pacity on exercise* Ed. M Scherrer H. Huber Publishers, 1971

15. Johnson, R.L Taylor F.H. & DeGraff A.C. J Functional significance of low pulmonary diffusing capacity for carbon monoxide. *J G Invest 44* 789 1965

16 Jones, N.L Pulmonary gas exchange during exercise in patients with chronic airway obstruction. *Clin. Sci. 31* 39 1966.

17 Lim, T.P.K. (Ed.) *Cardiopulmonary function tests in linical medicine* Charles C. Thomas Publishers Springfield, Illinois, U.S.A., 1966.

18. Malmberg, R. Gas exchange in pulmonary tuberculosis. *Scand. J re p Dis. 47* 262, 1967

19 Malmberg R. Gas exchange in pulmonary tuberculosis. *Scand. J p Dis. 47* 277 1967

20. Mellemgaard, K. The alveolar-arterial oxygen difference its size and components in normal man. *Act physiol scand 67* 10 1966.

21 Mellemgaard, K. The alveolar-arterial oxygen difference in chronic obstructive lung disease. *Scand J re p Dis 48* 23 1967

22. Piiper J., Unequal distribution of pulmonary diffusing capacity and the alveolar arterial P_{O_2} differences: theory. *J. appl. Physiol.* *16* 493 1961

23. Piiper J.: Variations of ventilation and diffusing capacity to perfusion determining the alveolar — arterial O_2 difference: theory. *J. appl. Physiol.* *16* 507 1961

24. Raine J.M. & Bishop J.M. A—a difference in O_2-tension and physiological dead space in normal man. *J. appl. Physiol.* *18* 284 1963.

25. Read, J & Williams, R.S. Pulmonary ventilation. Blood flow relationships in interstitial disease of the lungs. *Amer. J. Med.* *27* 545, 1959

26. Riley R.L. & Cournand, A. Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: theory. *J. appl. Physiol.* *4* 77 1951

27. Ross, J.C., Frayser R. & Hickman, J.B. A study of the mechanism by which exercise increases the pulmonary diffusing capacity for carbon monoxide. *J. Clin. Invest.* *38* 916 1959

28. Roughton, F.J.W. & Forster E.R. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lungs with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J. appl. Physiol.* *11* 290 1957

29. Said, S.I. Thompson W.T. Patterson, J.L. & Brummer D.L. Shunting effect of extreme impairment of pulmonary diffusion. *Bull. Johns Hopkins Hosp.* *105* 255 1960

30. Staněk, V. Widimsk J. Kasalický J. Navaratil M. Daum S. & Levinsky L. The pulmonary gas exchange during exercise in patients with pulmonary fibrosis. *Scand. J. resp. Dis.* *48* 11 1967

31. Staub N.C. Alveolar-arterial oxygen tension gradient due to diffusion. *J. appl. Physiol.* *18* 673 1963

32. Vale J.R. Pulmonary gas exchange in normal subjects at rest and during moderate exercise. *Scand. J. resp. Dis.* *48* 394, 1967

33. Vale, J.R. Pulmonary gas exchange and venous-arterial shunt in normal subjects breathing oxygen, at rest and during exercise. *Scand. J. resp. Dis.* *51* 305, 1970.

34. Vale J.R.: Effect of exercise and O_2 breathing on gas exchange in chronic bronchitis and in diffuse, restrictive pulmonary disease. *Scand. J. resp. Dis.* *51* 316 1970.

35. West, J.R. & Alexander J.K.: Studies on respiratory mechanics and the work of breathing in pulmonary fibrosis. *Amer. J. Med.* *27* 529 1959

CHAPTER V

CENTRAL HEMODYNAMICS IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

One feature of chronic lung disease is that it may cause structural or functional changes in the pulmonary vascular bed, leading to pulmonary hypertension and subsequently right ventricular hypertrophy (cor pulmonale) (46). In the definition of cor pulmonale (46) it is specially pointed out that cases with disease of the left heart should be excluded as the increase in pulmonary artery wedge pressure also contributes to the elevation of pulmonary artery pressures, although it does not change the pressure gradient across the pulmonary vascular bed (21, 27). The aim of the present investigation was to study

1. Central hemodynamics in terms of pressure flow relationships in patients with obstructive and restrictive lung disease.

2. The factors that contribute to the elevation of the pulmonary artery mean pressure — pulmonary wedge pressure difference, i.e. the pulmonary vascular resistance at rest and during exercise in these patients.

3. An attempt was also made to investigate the general effects of obstructive and restrictive dysfunction on the central hemodynamics.

Procedure

On the day of catheterization the patients had a light breakfast early in the morning and received a sedative two hours before the examination. Right heart catheterization was performed in the recumbent position as described earlier from this laboratory (22, 32). A double lumen catheter was introduced percutaneously according to Jonsson (33) or after exposing an antecubital vein in the left arm. In three cases a single-lumen catheter was used. The catheter was advanced to the pulmonary artery and wedged in a peripheral branch, so that the pulmonary artery and wedge pressures were measured simultaneously. A teflon catheter was introduced percutaneously into the right brachial artery by the Seldinger technique. Measurements of pressures, cardiac output and blood gases were made at rest and during work; the work load for each patient was approximately 2/3 of the working capacity as judged from a previously performed exercise tolerance test. Blood samples were drawn simultaneously from the pulmonary artery and brachial artery for one minute in the middle of the gas collection period for

Table 1 Catheterization data at rest and during exercise in group A. 16 men and 8 women with $\dot{V}o_2$ = oxygen ptake, Sa_{O_2} = oxygen saturation of the arterial blood, $S\bar{V}_{O_2}$ = oxygen satura- stroke volume RA = right atrium, RV = right ventricle PA = pulmonary artery $\overline{Pw}$ = pul- De = end diastolic, PVR = pulmonary vascula resistance index, SVR = systemic vascula re

Subject no.		Sex	Work kpm/min	$\dot{V}O_2$ ml STPD/min	Sa_{O_2} %	$S\bar{V}_{O_2}$ %	O_2 capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/min
1	U.A.	M	est	271	94 8	74 1	17 70	37 6	7._1	83
			.30	848	94.2	56 5	18.09	69.2	12 23	1 4
2	K.E.	M	rest	258	91 7	68.2	16.88	40 7	6.34	75
			250	899	89 8	40.9	17.65	87.3	10 30	107
3	H.F	M	rest	267	95 1	68.9	18.94	50 8	5.26	85
			200	824	96.9	53 1	19.91	89 2	9.24	118
4	E.E.	M	rest	286	94 9	63 4	16.78	53.8	5.32	86
			100	604	9? 1	51 5	17 45	72.2	8.57	109
5	W.K.	M	rest	324	94 7	72 7	17 90	40 4	r	75
			500	1064	91 4	45 7	19 99	96.3		
6	R.K.	M	rest	273	97.8	77 7	19 12	40 4		
			600	1272	9 6	43.0	20.77	101.0		
7	S J	M	rest	280	92 8	72 5	18 91	39 4		
			400	1078	94 1	49 5	19 40	87 6		
8	W M.	M	est	315	86.0	61 4		47 1		
			150	779	85 8	45		82 1		
9	G F	M	rest	220	93.8	70		40.2		
			200	428	93 5	42_		83.5		
10	H M	M	rest	240	94.2	63.5		.3		
			—	—	—	—				
11	E F	M	es	272	91.3	61.3				
			116	803	89.2	38 7				
1	[illegible]	M	est	238	49.9	41 1				
			116	547	54 4	27.8				
13	[illegible]	M	est	245	88.6	66.2				
			116	547	81.8	45 9	20			
14	V E	M	est	2 1	88 9	69 0	19.5			
			116	44	66 8	54 5	20 76			
15	[illegible]	M	es	47	93 4	68 8	17 43			
			)	8 6	96 6	45 0	18.38			
16	P F	M	es	1	91 3	65 4	14.87			
			130	8	89 4	39 4	15 40			

chronic obstructive lung disease.
tion of mixed venous blood, AVD = arteriovenous oxygen difference, HR = heart rate, SV =
monary mean wedge pressure Br_A = brachial artery S = systolic, D = diastolic, M = mean,
sistance index.

SV ml	RA	RV		Pressures, mm Hg			$\overline{P}w$	BrA			PVR	SVR
				PA								
		S	Do	S	D	M		S	D	M		
87	0.3	24	2	4	10	17	6	131	73	95	2.4	21.0
99		38	4	31	12	24	8	142	78	103	2.1	13 4
85	2	25	2	23	10	16	5	126	76	84	3 8	23.2
96		48	4	45	21	29	7	161	92	118	4 0	20 0
6	4	23	6	20	9	15	9	147	77	100	2.0	32.7
78		55	14	47	24	34	22	184	94	131	2	4 4
62	5	29	7	29	14	20	8	120	103	132	4.2	48.3
77		48	11	46	23	34	14	219	116	165	4 4	36.5
110	5	29	6	28	10	18	11	125	59	84	1 7	20 1
84		62	11	63	25	45	32	156	72	114	2 3	19.8
90	3	22	4	20	9	15	7	127	77	98	3	28 2
82		45	3	33	12	22	6	188	92	129	6	20 6
96	6	27	10	26	11	18	11	110	69	90	1 9	24 6
120		40	12	48	23	35	22	152	82	113	2.1	17.8
86	8	32	10	29	14	21	10	140	90	111	3 4	34.2
98		40	17	39	23	34	21	162	102	134	2 8	29 1
72	0	1	4	24	9	15	6	103	45	76	2.8	23 6
50		43	11	4	19	29	1	164	78	116	5 7	38 4
60	4	[illegible]	4	[illegible]	10	17	6	114	65	83	4 0	30 4
—	—	—	—	—	—	—	—	—	—	—	—	—
56		26	3	29	11	19	8	149	78	108	4.5	42.9
73		49	15	47	21	32	16	196	90	144	3 8	33 1
117	7	82	8	57	24	38	10	102	61	81	4.2	11.9
73		88	18	88	34	5	16	140	80	102	7 1	19.9
58	2	4		26	15	19	5	135	76	97	4.8	34.2
73		54	5	56	30	39	10	163	89	122	5 4	29 7
58	3	36	6	36	19	26	10	133	77	109	4 7	32.2
68		80	17	69	35	30	17	180	103	132	6.3	26.0
63	4	4	4	24	10	16	6	147	96	116	3.3	37 4
79		43	9	41	23	29	10	175	99	130	3.9	23 6
53	6	27	6	29	16	20	12	110	70	92	3.0	34.2
92		49	13	48	23	32	21	119	61	86	1.9	15.1

Subject no		Sex	Work kpm/min	$\dot{V}_{O_2}$ ml STPD/min	S_{aO_2} %	$S\bar{V}_{O_2}$ %	O_2 capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/min
17	S.E.	F	rest	224	94.9	69.3	17.67	46..	4.84	57
			150	673	94 1	46.4	18.93	90 6	7 43	93
18	E.K.	F	rest	212	91.9	71.9	16 47	34 0	6.24	77
			100	554	92.6	45.2	16.92	81.2	6.82	96
19	E.A.	F	rest	[illegible]05	98.4	69 7	16.71	49.9	4 11	76
			100	570	89 4	50 1	17.31	69 1	8.23	97
20	B W	F	est	233	86.3	66 4	16 73	34 4	6 77	91
			200	639	76 6	59.8	18.25	67..	9.31	13[illegible]
21	A.F	F	est	211	95 4	70.1	15 97	41.3	5.08	63
			150	567	91.3	50.3	17.34	72.1	7.86	92
22.	E.P	F	rest	180	94.0	74.3	15 81	31 7	5 68	74
			250	709	91.9	44.6	16 11	77 2	9 18	116
23	A.S	F	rest	169	94.0	67.2	15.89	43 6	3 88	80
			—	—	—	—	—	—	—	—
24	L.H.	F	rest	233	91.9	68.9	18.01	42 4	5.54	115
			116	613	89.8	47.2	17.92	77 3	7 93	129

the determination of arterio-venous oxygen difference (AVD) From the brachial artery a blood sample was collected as well immediately after the measurement of the cardiac output, for the determination of Pa_{O_2}, Pa_{CO_2} and pH All examinations were carried out without complication except in patient 7 group B who developed a transient complete heart block during exercise in conjunction with measurement of the cardiac output

Results

Individual ath [illegible] n data are shown in Tables I II Ex [illegible] measurements only f [illegible]2 of t[illegible] [illegible] patient in group A and 16 of 1[illegible] [illegible] group B are available, as two patients in the first group and one in the second had to interrupt the exercise shortly after the start because of dyspnea.

In group A the *mean work load* was 208 kpm/min, range 100—600 and in group B 252 kpm/min, range 116—450

The oxygen uptake in group A was at rest 240 ml/min, SD ± 41 and during exercise 731 ml/min, SD ± 210 In group B the values at rest and during exercise were 258 ml/min, SD ± 36 and 848 ml/min, SD ± 234 respectively

The heart rate in group A averaged at rest 81 beats/min and during exercise 111 beats/ [illegible] group B the average was [illegible] and 111 beats/m[illegible]

SV ml	RA	RV		PA			$\overline{P}_w$	Br_A			PVR	SVR
		Pressures, mm Hg										
		S	De	S	D	M		S	D	M		
85	4	31	7	33	15	21	10	130	73	95	3.6	30.6
80		53	9	50	23	37	11	160	88	120	5.5	25.2
81	3	42	6	32	16	24	7	154	86	113	4 4	29.0
71		49	9	54	23	39	14	185	94	133	5.9	31.2
54	1	24	3	33	15	23	9	158	83	114	5 0	41.0
85		70	11	75	30	45	18	180	83	127	3.9	22.8
72	2	39	6	36	17	26	10	142	81	107	3 6	24.2
72		65	11	60	33	44	24	178	99	135	3.2	21 7
78	2	24	3	23	7	13	4	117	69	93	3.5	29 4
85		44	10	39	17	27	11	138	84	107	3 3	21 9
77	4	25	7	24	8	16	10	112	59	88	1 5	21 7
79		41	11	41	18	29	14	154	79	111	2.3	16.9
49	4	26	6	29	13	18	14	127	69	99	1.5	35 7
—	—	—	—	—	—	—	—	—	—	—	—	—
48	4	29	8	26	10	18	11	121	83	103	2.2	32.9
61		34	8	30	13	21	12	140	82	110	2.0	24.6

In group A the *arterial oxygen saturation* was 91.2 % at rest, which is slightly lower than normal, and decreased during exercise to 87.9 % $p < 0.05$. The lowest value at rest was 49 9 % found in patient A 12 with secondary polycythemia and severe alveolar hypoventilation with arterial P_{CO_2} of 64 mm Hg. In group B Sa_{O_2} was 94.5 % at rest and decreased during exercise to 90.9 % $p < 0.05$

The oxygen saturation of the mixed venous blood was in group A 67 7 % at rest and 44.5 % during exercise. In group B it was 70.0 % at rest and 46.3 % during exercise

The arterio-venous oxygen difference in group A was at rest 43.5 ml/l and during exercise 84 2 ml/l. In group B it was 43 1 ml/l at rest and 85 2 ml/l during exercise.

The oxygen tension in arterial blood in group A was at rest 64 mm Hg, range 32—93 and during exercise 61 mm Hg, range 35—88 ($p > 0.05$). In group B it was 72 mm Hg, range 63—83 at rest and decreased slightly during exercise ($p < 0.05$) to 66 mm Hg, range 53—83

The carbon dioxide tension in arterial blood in group A was at rest 43 mm Hg, range 31—69 and during exercise 44 mm Hg, range 32—68. Only 4 patients had elevated $Pa_{CO_2} > 45$ mm Hg at rest and during exercise. The corresponding values in group B were 39 mm Hg, range 34—46 at rest and 40 mm Hg, range 30—47 during exercise.

Table II Catheterization data at rest and during exercise in group B. 12 men and 5 women with

Subject no		Sex	Work kpm/min	V_{O_2} ml STPD/min	$S_{a_{O_2}}$ %	$S\bar{v}_{O_2}$ %	O_2 capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/min
1	H.B.	M	rest	280	96.8	69.2	17.39	50.0	5.60	85
			300	705	96.2	48.0	17.97	88.6	7.96	108
2.	E.E.	M	rest	259	98.5	80.9	20.69	38.4	6.74	88
			200	707	95.6	64.8	20.13	63.0	12.17	110
3	R.U	M	rest	336	93.2	70.2	17.26	40.7	8.26	99
			300	1124	91.2	47..	17.83	84.7	13.27	135
4	V.S.	M	rest	267	93.0	63.3	17.17	51.0	5.24	81
			250	838	72.9	27.7	17.58	79.5	10.54	114
5	J.S	M	rest	277	9 .9	74.2	18.00	40.0	6.93	75
			450	1214	91.1	44.5	18.97	89.4	13.91	125
6	B.E.	M	rest	268	92.5	63.8	12.38	36.5	7.34	80
			250	915	85.7	29.8	24.15	79.1	11.57	108
7	O.Z.	M	rest	270	93.7	67.1	15.18	44.4	6.08	76
			150	714	94.1	32.5	16.3.	101.7	7.0.	60
8	L.O	M	rest	269	93.4	62.0	16.59	53.1	5.07	78
			250	810	86.8	40.6	17.13	80.2	10.47	114
9	L.P	M	rest	227	94.8	71.5	18.41	43.9	5.17	79
			300	1038	91.8	46.3	18.81	86.6	11.99	95
10.	O.M	M	rest	285	97.1	72.3	18.91	48.9	5.83	78
			200	791	96.6	46.4	19.70	100.9	7.84	103
11	I.H	M	rest	42	93.3	63.9	17.38	52.1	4.61	91
			—	—	—	—	—	—	—	—
1	A.R.	M	rest	307	93.0	70.5	18.91	43.4	7.04	70
			400	1196	94.4	42.9	20.06	104.3	11.47	11
13	C.J	F	rest	235	90.3	71.7	17.73	33.2	6.91	73
			400	1103	83.0	68.6	18.73	—	—	138
14	S.H	F	rest	221	90.9	65.6	18.40	47.6	4.60	67
			1 .0	537	—	53.0	19.07	82.2	6.80	91
15	H.B	F	rest	221	92.0	73.7	16.15	30.6	7.22	102
			116	476	88..	56.0	16.88	56.1	8.48	122
16.	A.D	F	rest	1 3	97.8	73.1	16.13	41.9	4.61	86
			?00	6	96.9	43.5	16.87	92.1	6.47	114
17	I.P	F	r	3		73.4	17.73	36.8	6.06	96
			116		4	48.4	19.10	89.9	8.08	130

chronic restrictive lung disease. Symbols as in Table I

SV ml	RA	RV S	RV De	PA S	PA D	PA M	$\overline{Pw}$	BA S	BA D	BA M	PVR	SVR
		Pressures, mm Hg										
66	1	28	4	31	13	19	7	128	55	81	3.7	24 6
74		48	8	48	23	30	6	185	70	114	5.1	24 4
77	3	26	6	23	7	14	8	117	76	81	1 7	23 7
111		42	6	42	16	29	10	142	89	103	3 0	16.1
83	3	31	7	30	13	20	6	133	82	103	3 0	21.8
98		46	7	46	20	33	13	161	92	124	2.8	16.4
65	3	55	9	54	28	37	15	138	81	100	7 1	32.5
92		85	19	92	48	65	22	153	82	110	6.9	17 7
92	5	35	8	32	12	21	10	115	71	90	3 0	24.1
113		46	12	53	19	35	17	158	84	119	2.4	15.9
92	4	1	7	23	10	16	8	128	87	109	1.9	25 7
107		53	13	58	21	39	20	169	103	136	2.8	20.3
80	3	37	9	33	12	22	8	153	73	100	4.2	29 6
117		68	18	68	32	52	33	205	114	149	4 0	35
63	0	22	1	22	5	13	0	132	79	102	4.9	38.2
92		49	9	40	13	26	4	169	93	132	4.0	24.0
65	9	39	9	36	17	27	12	130	79	102	5 7	39 4
126		66	17	59	23	42	17	179	90	131	4 1	21.8
73	5	42	9	41	16	26	16	161	77	114	3.6	41 0
73		70	19	68	35	51	37	174	91	107	3 8	28.7
49	3	36	8	29	11	19	8	139	69	98	3.9	33.0
—		—	—	—	—	—	—	—	—	—	—	—
101	5	26	8	24	10	18	10	130	78	104	2 2	28.5
102		4	11	40	14	28	13	167	92	119	2.5	20 0
95	6	25	3	29	12	21	11	109	67	87	2.7	23 8
—		50	9	53	20	36	11	13[illegible]	79	89	—	—
69	10	[illegible]5	7	23	13	18	9	112	69	91	3 2	32 0
73		32	7	28	15	22	13	110	65	84	2.2	21.2
71	0	29	4	27	12	20	7	137	72	110	3 0	23.3
70		43	9	42	19	31	14	141	79	109	3 1	19 8
54	5	19	4	21	8	13	8	93	58	79	1.7	27 4
57		33	6	34	15	24	13	117	70	92	2.7	22 8
63	3	21	2	20	6	12	6	130	72	99	1 7	27 4
62		50	10	4[illegible]	23	34	23	194	[illegible]6	144	2.3	29.9

pH in arterial blood in group A was at rest 7 41 range 7.30—7 48, and during exercise 7.38 range 7.28—7.51 and in group B at rest 7 42, range 7.37—7 49 and during exercise 7 39 range 7.31—7 48 Among the 4 patients in group A with elevated Pa_{CO_2} only one (A 12) had a pH lower than 7.35 the other three patients had normal pH

Intracardiac and intravascular pressures The individual values are given in Tables I and II mean values ± SD are given in Table III

At rest The mean pressures were compared with mean values reported in healthy elderly subjects aged 61—83 years by Granath *et al* (22) and Granath and Strandell (23) In group A the systolic and end diastolic pressures in the right ventricle (RV_s, RV_D) pulmonary mean wedge pressure ($\overline{P}_W$) and the brachial artery systolic (Br_{A_s}) diastolic (Br_{A_d}) and mean $\overline{P}Br_A$) pressures were not significantly different (p >0 05) from normal values. The pulmonary artery systolic (P_{PA_s}) diastolic (P_{PA_d}) and mean ($\overline{P}_{PA}$) pressure were slightly higher (p <0 05) In group B P_{PA_s} and $\overline{P}_{PA}$ were slightly higher than normal (p < 0 05) while other pressures were not significantly different (p >0 05)

During exercise As the work load in this material differed from that for the healthy elderly subjects mentioned above, the regression lines of the pressures on the cardiac output including the values both at rest and during exercise) were compared in the two materials (Figs 1 3) In both groups the regression line of RV had a slope that was not significantly different from that in normal subjects (p >0.05) The intercept was lower in group A (p <0.01) in group B higher (p < 0 05) The regression lines of RV_D in both groups were identical with that in normal subjects (Fig 1)

The $\overline{P}_{PA}$ regression line had approximately the same slope as in healthy subjects (p >0 05) but the intercept was significantly higher in group A (p <0.001) and in group B (p < 0 01) Fig 2 In both groups $\overline{P}_W$ values during exercise were within normal limits except in two patients in group B (Nos. 7 and 10) and in one patient in group A (No. 5) for whom it was higher than + 2 SD These patients had signs of myocardial disease. Two other patients, one from each group, had a lower P_W than —2 SD The slope of $\overline{P}_W$ on the cardiac output in group B was slightly lower than in normal subjects (p <0 05) while the intercept was not significantly different (Fig 3) In group A no significant difference was found in slope or intercept compared with normal subjects. The regression of Br_A on the cardiac output was in both groups not significantly different from the regression in normal subjects (Fig. 4 and 5) However most of the pressures in both groups showed a wide individual variation from normal to highly elevated.

As $\overline{P}_{PA}$ was elevated in the two groups both at rest and exercise, while $\overline{P}_W$ was normal, the difference between the two pressures ($\overline{P}_{PA}$—$\overline{P}_W$) was significantly larger in the patient material than in nor

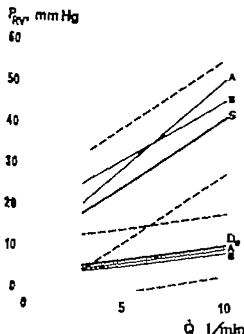


Fig 1 Regression of right ventricular systolic (S) and end diastolic (De) pressures on cardiac output (Q) t rest and during exercise in group A and in group B. Thick solid lines and interrupted lines are regression lines ± 2 SD for healthy elderly subjects (22, 23)

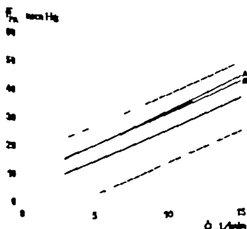


Fig 2 Regression of pulmonary artery mean pressure ($\overline{P}_{PA}$) on cardiac output at rest and during exercise. Material and symbols as in Fig 1

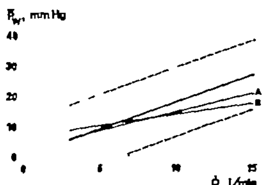


Fig 3 Regression of mean wedge pressure ($\overline{P}_W$) on cardiac output t rest and during exercise. Material and symbols as in Fig 1

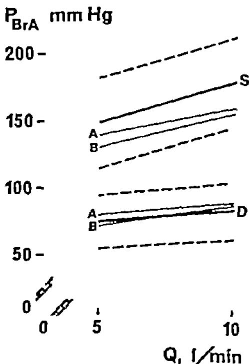


Fig 4 Regression of brachial artery systolic (S) and diastolic (D) pressures on cardiac output rest and during exercise Material and symbols as in Fig 1

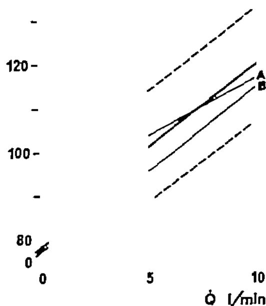
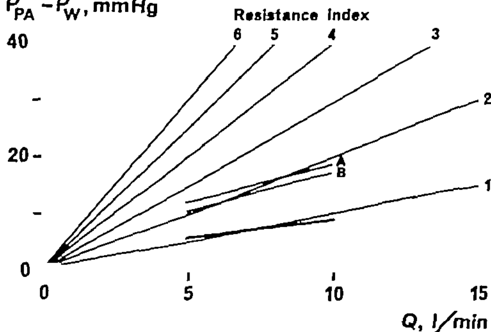


Fig 5 Regression of brachial artery mean pressure ($\overline{P}_{BrA}$) on cardiac output t rest and during exercise Material and symbols as in Fig 1

Fig 6 Regression of the pressure difference ($\overline{P}_{PA}-\overline{P}_{W}$) on cardiac output at rest and during exercise. Material and symbols as in Fig. 1



mal material (Fig 6) $\bar{P}_{PA}-\bar{P}_W$ was identical in the two groups and averaged at rest 11 mm Hg, increasing significantly during exercise to an average of 19 mm Hg The mean vascular pressures in groups A and B are given in Table III. There was no significant difference between the group means as regards these pressures either at rest or during exercise. Also there was no significant difference between the slopes of pressures on the cardiac output at rest and exercise in the two groups.

The pulmonary vascular resistance index was, in group A, 3.3 units at rest and increased slightly during exercise to 3 9 units ($p < 0.05$) In group B the average was 3 4 units at rest and 3.5 units during exercise ($p > 0.05$) The values were significantly higher than values reported in healthy elderly subjects (23)

Parameters for the pressure difference ($\bar{P}_{PA}-\bar{P}_W$)

$\bar{P}_{PA}-\bar{P}_W$ at rest in group A was significantly correlated with Sa_{O_2} ($r = -0.803$) Pa_{O_2} ($r = -0.731$) and Pa_{CO_2} ($r = 0.782$) in linear regression analysis (for all values $p < 0.001$) A significant correlation was also found during exercise with Sa_{O_2} ($r = -0.681$) Pa_{O_2} ($r = -0.660$)) ($p < 0.001$) and Pa_{CO_2} ($r = 0.624$) ($p < 0.01$) No significant correlation was found with pH either at rest or during exercise. In group B a significant correlation was found between $\bar{P}_{PA}-\bar{P}_W$ and Sa_{O_2} during exercise only ($r = -0.807$) ($p < 0.01$) and with Pa_{O_2} ($r = -0.638$) ($p < 0.05$) At rest and during exercise no significant correlation was found with Pa_{CO_2} and pH (Table IV)

In group A the pulmonary artery diastolic pressure — pulmonary mean wedge pressure difference ($P_{PAd}-\bar{P}_W$) was significantly correlated with Sa_{O_2} and Pa_{CO_2} both at rest and during exercise (Table IV) In group B there was a significant correlation during exercise only The correlation coefficients for blood gases with $P_{PAd}-\bar{P}_W$ were lower than those with $\bar{P}_{PA}-\bar{P}_W$ In neither group was a correlation found with pH nor was the pulmonary mean wedge pressure correlated with Sa_{O_2} Pa_{CO_2} or pH.

In group A the *cardiac output* in men at rest was 6 18 l/min, SD $\pm$ 1 64 $n = 16$ and during exercise 9 34 l/min, SD $\pm$ 2.00 $n = 15$ The corresponding cardiac indexes were 3 50 l/m² $\pm$ 1 03 and 5 25 l/m $\pm$ 1 11 In women the figure at rest was 5 27 l/min, SD $\pm$ 0 99 $n = 8$ and during exercise 8.14 l/min, SD $\pm$ 0.94 $n = 7$ and the corresponding cardiac indexes were 3 42 l/m² $\pm$ 0 62 and 5 25 l/m $\pm$ 0 89 The relationship between cardiac output and oxygen uptake at rest and during exercise (Fig 7) was within $\pm$ 2 SD for the regression line in healthy elderly subjects (22) except in one patient (A 12) in whom it was higher than + 2 SD He had severe hypoxemia. The relationship between cardiac output and oxygen uptake for all observations both at rest and during exercise followed the equation $Q = 4.20 + 6.6 \dot{V}_{O_2}$ $r = 0.851$ SD $\pm$ 1 20 $n = 46$ $p < 0.001$ The regression of the

T bl III Mean pressures ± SD in group A and in group B at rest (R) nd during exercise (E)
For symbols, see text.

			RV		PA			$\overline{P}_W$	BrA			$\overline{P}_{PA}$—$\overline{P}_W$
			S	De	S	D	M		S	D	M	
A	R	n=24	29.8±12.3	5.5±2.3	28.5±7.5	12.6±4 0	19.5±5 2	8.5± 7	131.3±20.0	74.8±12.5	99 0±14 1	10 7±5.0
	E	n=22	52 1±13 5	10.7±4.3	30 0±14.0	23.0±6.5	34 6±8.6	15 4±6.4	165.3±22.8	88 0±11.9	121.9±16.7	19.3±7.3
B	R	n=17	30.4±9.3	6.2±2 7	29.3±8.6	12 1±5.2	19 8±6.1	8.8±3.6	128.5±16.2	73.2±8.2	97.2±10 3	11.5±4 1
	E	n=16	51.6±14.0	11.3±4.6	51 1±15 6	22.3±9 1	36.1±11.5	16 6±8.8	159 9±26 0	86.2±12.8	116.4±19.2	19 4±8.0

T bl II Relationships between pressures and blood gases in group A and in group B t rest (R) and during exercise (E) For symbols, see text.

		$\overline{P}_{PA}$		P_{PA_d}		$\overline{P}_{PA}$ $\overline{P}_W$		P_{PA_d}-$\overline{P}_W$	
		R	E	R	E	R	E	R	E
Group A	SaO_2	—0.818	—0.716	—0.692	—0.691	—0.803	—0.681	—0.593	—0.565
	P	***	**	***	***		**		**
		21	22	24	22	24	22	24	22
	$PaCO_2$	0 808	0.704	0.709	0.716	0.782	0.674	0 600	0 446
	P	***	**	**	***	**		**	*
		[illegible]	22	24	22	24	22	24	22
Group B	SaO_2	—0 175	—0.506	—0.202	—0 461	—0.292	—0.807	—0.339	—0.645
	P	—		—	—	—	**	—	**
		17	15	17	15	17	15	17	15
	$PaCO_2$	0.231	—0.153	0.256	—0 153	0.407	0.513	0.427	0.659
	P	—		—	—	—	—	—	
	n	14	13	14	13	14	13	14	13

cardiac output on oxygen uptake had approximately the same slope as in healthy young subjects (13) but the intercept was obviously lower (Fig 9) However neither slope nor intercept differed when compared to the regression line found in healthy elderly subjects (22)

In group B the cardiac output in men at rest was 6 16 l/min, SD ± 1 10 n = 12, and during exercise 10 74 l/min, SD ± 2.27 n = 11 The corresponding cardiac index was 3.37 l/m² ± 0 65 and 5 83 l/m ± 1.29 In women the cardiac output was at rest 5.88 l/min, SD ± 1.24 n = 5 and during exercise 7 46 l/min, SD ± 0.97 n = 4 the corresponding cardiac index being 3.54 l/m² ± 0 75 and 6.64 l/m² ± 0.67 In one female patient the cardiac output during exercise was discarded. At rest and during exercise the relationship between the cardiac output and oxygen uptake was within ± 2 SD except in patient B 2 whose cardiac output during exercise was >2 SD (Fig 8) The regression of cardiac output on the oxygen uptake at rest and during exercise was according to the following equation $Q = 4.12 + 7.1\ V_O$ $r = 0.886$ SD ± 1 26 n = 32, $p < 0.001$ The regression line had a slope of 7 1 compared to 6.6 in group A, a difference which was not significant ($p > 0.05$) Also the intercept was approximately the same in both groups (Fig 9)

In group A the *stroke volume* in men at rest was 76.1 ml, SD ± 20.2 n = 16 and during exercise 82.8 ml, SD ± 16.5 n = 15 in women 68.0 ml, SD ± 15 1 n = 8 at rest and 76.1 ml, SD ±

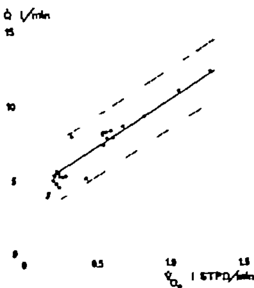


Fig 7 Cardiac output in relation to oxygen uptake at rest (n = 24 open symbols) and during exercise (n = 22, solid symbols) in patients with obstructive lung disease. Regression line ± 2 SD for healthy elderly subjects (22, 23)

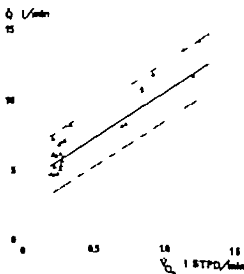


Fig 8 Cardiac output in relation to oxygen uptake at rest (= 17 open symbols) and during exercise (= 15 solid symbols) i patients with restrictive lung disease Regression line ± 2 SD as in Fig. 7

8 7 n = 7 during exercise There was no significant difference between females and males either at rest or during exercise (p >0 05) The average increase in stroke volume when only paired observations were included was 8 % in men and 7 % in women. The mean values at rest and during exercise were significantly lower than values reported in healthy young subjects by Bevegård *et al* (4) and Holmgren *et al* (3?) The stroke volume in men at rest did not differ significantly from values reported in healthy elderly subjects by Granath *et al.* (22) but during exercise it was significantly lower The stroke volume in relation to THb and HV during supine exercise was in most of the patients lower than —2 SD of the regression line found in healthy young subjects (4 5 31 32) The relationship between the stroke volume and THb is shown in Fig. 10 The stroke volume during exercise was positively correlated with THb and PVR ($p < 0.05$) but not significantly with BV HV $\bar{P}_{PA}$ $\bar{P}_{PA}$—$\bar{P}_W$ or Sa_{O_2}

In group B the stroke volume in men was at rest 78 3 ml, SD ± 1? 7 n = 11 and during exercise 100.6 ml, SD ± 16.5 n = 11 In women the values were 64 3 SD ± 7 6 n = 4 at rest and 66 ml SD ± 8 n = 4 during exercise The increase in the stroke volume between rest and exercise averaged in men 28 and in women 3 % The relationship between the stroke volume during exercise and THb and HV was within ± ? SD except in two males and three females, who had small stroke volumes in relation to their THb and HV The relationship between the stroke volume and THb is shown in Fig 11 One of

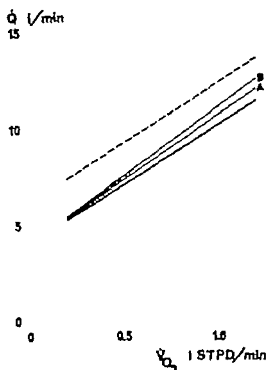


Fig 9 Regression lines for cardiac output on oxygen uptake at rest and during exercise in group A and in group B compared with regression lines for healthy elderly (solid line) and young (interrupted line) subjects

these male patients (B 4) had left bundle branch block and the other (B 10) had ST depression in his exercise ECG The three females had normal heart volumes in relation to total amount of hemoglobin. In the fifth female patient (B 13) the stroke volume, measured only at rest was 95 ml, which was normal in relation to THb and heart volume. In group B the stroke volume during exercise was significantly correlated with THb ($p < 0.05$) but no correlation was found with BV HV $\bar{P}_{PA}$ $\bar{P}_{PA}$—$\bar{P}_W$ PVR, Sa_{O_2} or VC % of predicted. In men the stroke volume during exercise was significantly larger



Fig 10 Stroke volume during supine exercise in relation to total amount of hemoglobin of women (open symbols) and 15 men (solid symbols) with obstructive lung disease. Regression line ± 2 SD for healthy young subjects (4 5 31 32)



Fig 11 Stroke volume during supine exercise in relation to total amount of hemoglobin of 4 women (open symbols) and 11 men (solid symbols) with restrictive lung disease Regression line ± 2 SD as in Fig. 10.

in the restrictive than in the obstructive group ($p < 0.02$) but no significant difference was found at rest ($p > 0.05$) In women there were no significant differences between the two groups either at rest or during exercise. This might be due to the small number of female patients in the restrictive group

In order to demonstrate the effect of the degree of airway obstruction on the hemodynamics, group A was divided into 3 subgroups according to the FEV % (of predicted) Table V Subgroup I included patients with FEV % more than 75 % mean 82 % n = 4 subgroup II with FEV % 51—75 % mean 65% n = 10 and subgroup III with FEV % ≤50 % mean 47 % n = 10 Patient 14 A.E., was included in subgroup III as he had a very low MVV (15 l/min) although FEV % was 61 % of the predicted value.

At rest and during exercise with increasing grade of airway obstruction (decreasing FEV %) there was a decrease in Sa_{O_2} without significant difference between the subgroup means. There was a correlation of probable significance between FEV and Sa_{O_2} ($p < 0.05$) for the whole group during exercise, but no significant correlation was found at rest. Also FEV was linearly correlated with

T W V The relative effects of obstructi entilatory dysfunction upon central hemodynamics.

	Sa_{O_2}		Pa_{CO_2}		$\overline{P}_W$	
	R	E	R	E	R	E
I FEV % > 75 % predict.	95.2±1.8 (4)	91.5±1 7 (4)	40.0±2.9 (4)	39.8±4 6 (4)	10.0±2.2 (4)	18.5±11.0 (4)
II FEV % 51—75 % predict.	94.8±1.5 (10)	93 6±2.4 (9)	38.8±5.7 (10)	40.4±5.8 (9)	8.3±2.9 (10)	14.2±5.2 (9)
III FEV % ≤ 50 % predict.	86.1±13 0 (10)	80.7±13.0 (9)	47.3±10.3 (10)	49 1±12.1 (9)	8.0±2.7 (10)	15.2±5.3 (9)

T ble V I The relati effects of restricti entilatory dysfunction upon central hemodynamics.

	Sa_{O_2}		Pa_{CO_2}		$\overline{P}_W$	
	R	E	R	E	R	E
(a) VC 63 % pred range 51—72	94.2±2.2 (9)	91 1±8.1 (8)	37.0±3 3 (7)	37 4±5.3 (7)	9 4±3 7 (9)	20.0±10.1 (9)
(b) VC 41 % pred range 29—47	91.8±2.8 (8)	90.5±12.2 (7)	40.4±4 1 (7)	42.6±4.8 (7)	8 0±3 7 (8)	12.5±4.5 (7)

Pa_{O_2} both at rest and during exercise ($p < 0\ 05$) Subgroup III had slightly higher Pa_{CO_2} than subgroup II at rest ($p < 0\ 05$) but not during exercise ($p > 0\ 05$) There was no significant linear correlation between FEV_1 and Pa_{CO_2} for the whole group either at rest or during exercise

The pulmonary artery mean pressure ($\overline{P}_{PA}$) was 5 mm Hg higher in subgroup III than in subgroup I at rest and 6 mm Hg during exercise but there was hardly any difference between subgroups I and II The pulmonary mean wedge pressure ($\overline{P}_W$) did not show any significant difference between the subgroups either at rest or during exercise. Thus the pressure difference ($\overline{P}_{PA}—\overline{P}_W$) increased with decreasing FEV % (Fig 12) At rest a significant difference in $\overline{P}_{PA}—\overline{P}_W$ was found between subgroups I and III and between subgroups II and III ($p < 0\ 05$) During exercise there was an increase from 13.8 mm Hg in subgroup I to 22.6 mm Hg in subgroup III FEV_1 was negatively correlated with $\overline{P}_{PA}—\overline{P}_W$ both at rest and during exercise ($p < 0.05$) It followed that the pulmonary vascular resistance (PVR) at rest showed a significant difference between subgroups I and III ($p < 0.01$) and between subgroups II and III ($p < 0.05$)

Mean values ± SD number of observations in parentheses.

$\bar{P}_{PA}$ R	$\bar{P}_{PA}$ E	$\bar{P}_{PA}-\bar{P}_{W}$ R	$\bar{P}_{PA}-\bar{P}_{W}$ E	C.I. R	C.I. E	PVR R	PVR E
17.3±2.2 (4)	32.0±9.6 (4)	7.3±1.0 (4)	13.8±[illegible] (4)	3.60±0.68 (4)	6.0[illegible]±0.42 (4)	2.1±0.7 (4)	[illegible]3±0.3 (4)
17.6±3.1 (10)	32.7±6.3 (9)	9.3±3.0 (10)	18.4±5.2 (9)	3.22±0.52 (10)	5.24±1.29 (9)	2.9±1.1 (10)	3.8±1.4 (9)
22.4±6.6 (10)	37.8±10.0 (9)	14.4±5.7 (10)	22.6±9.1 (9)	3.70±1.23 (10)	4.90±0.74 (9)	3.8±0.7 (10)	4.7±2.0 (9)

Mean values ± SD number of observations in parentheses.

$\bar{P}_{PA}$ R	$\bar{P}_{PA}$ E	$\bar{P}_{PA}-\bar{P}_{W}$ R	$\bar{P}_{PA}-\bar{P}_{W}$ E	C.I. R	C.I. E	PVR R	PVR E
20.9±7.2 (9)	39.3±15.9 (9)	11.4±4.8 (9)	19.3±10.0 (9)	3.58±0.62 (9)	5.30±1.35 (9)	3.4±1.6 (9)	3.6±1.6 (9)
18.5±4.9 (8)	31.9±6.3 (7)	10.5±3.5 (8)	19.6±5.0 (7)	3.32±0.72 (8)	5.83±1.14 (6)	3.3±1.4 (8)	3.2±0.7 (6)

During exercise the difference was mainly between subgroups I and III ($p < 0.01$). The correlation between FEV_1 and PVR for the whole group was significant at rest ($r = -0.546$, $p < 0.01$) and during exercise ($r = -0.506$, $p < 0.05$). The cardiac output was not influenced by airway obstruction either at rest or during exercise. The stroke volume averaged in subgroups I—III at rest 48, 43 and 42 ml/m² and the corresponding values during exercise were 55, 49 and 44 ml/m². There was no significant difference between the subgroup means ($p > 0.05$). There was no significant correlation between FEV and the stroke volume either at rest or during exercise ($p > 0.05$).

To study the effect of restrictive ventilatory dysfunction on the hemodynamics, group B was divided into two subgroups (a) VC > 50 % of predicted, mean 63 %, n = 9, mean age 58 years, and (b) VC < 50 % of predicted, mean 41 %, n = 8, mean age 50 years (Table VI). Sa_{O_2} did not show a significant difference between the subgroups. There was no significant correlation between Sa_{O_2} or Pa_{O_2} and VC either at rest or during exercise. But there was a slightly significant negative correlation between Pa_{CO_2} and VC both at rest and during exercise ($p < 0.05$). Pa_{CO_2} was slightly higher in (b) than in (a). At rest $\bar{P}_{W}$

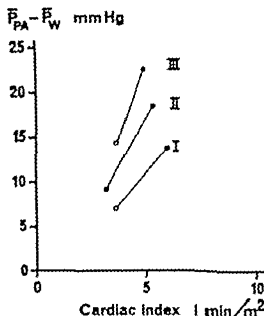


Fig. 12 Mean pressure difference $\overline{P}_{PA}$—$\overline{P}_W$ in relation to cardiac index at rest and during exercise in patients with obstructive lung disease

I patients with FEV % (of predicted) >75 %

II patients with FEV % (of predicted) 51—75

III patients with FEV % (of predicted) ≤50 %

and $\overline{P}_{PA}$ did not show a significant difference between subgroup (a) and subgroup (b). During exercise $\overline{P}_W$ measured 20 mm Hg in subgroup (a) and 12.3 mm Hg in subgroup (b) and $\overline{P}_{PA}$ measured 39.3 mm Hg in (a) and 31.9 mm Hg in (b). The difference was not significant (p >0.05). The $\overline{P}_{PA} - \overline{P}_W$ during exercise was virtually identical in (b) and (a). The cardiac output and the stroke volume were not affected by the decrease in vital capacity.

Discussion

Chronic lung disease causes changes in the pulmonary circulation through structural or functional changes of the pulmonary vessels, leading to an increased vascular resistance and elevation of pulmonary artery pressure. An elevation in $\overline{P}_W$ may contribute to the increase of pulmonary artery pressure (21, 27, 38). In both groups $\overline{P}_W$, normal at rest increased during exercise, contributing to about 50 % of the rise in the pulmonary artery mean pressure. It is preferable therefore to study pressure changes, particularly *during exercise, on the basis of* changes in the pressure gradient across the pulmonary vascular bed (21, 27, 35), as elevation of $\overline{P}_W$ does not contribute to the pressure gradient (27). This study demonstrated a slightly elevated pressure difference $\overline{P}_{PA}$—$\overline{P}_W$ at rest in both groups, which increased 8 mm Hg during exercise. It also demonstrated in the obstructive group a slight oxygen desaturation at rest, which increased slightly during exercise. In the restrictive group the oxygen saturation at rest was normal and decreased slightly during exercise.

Following the demonstration of pressure increase on induced hypoxia in cat (16) and in man (19, 40) many investigators confirmed the importance of hypoxemia as a factor in the genesis of pulmonary hypertension in chronic lung disease (1, 15, 24, 26, 43). The results of this study add further confirmation to the previous findings. The absence of a significant correlation at rest in the restrictive group was probably due to the normal oxygen saturation at rest in these

patients. In 23 patients of 43 with pulmonary fibrosis reported in the literature and reviewed by Wade and Bishop (44) the Sa_{O_2} was normal at rest. Also in patients with pleural restriction secondary to pulmonary tuberculosis the oxygen saturation at rest was found to be normal (39)

An increase in arterial P_{CO_2} has been implicated as a cause of pulmonary hypertension in patients with chronic lung disease when breathing carbon dioxide mixtures (20 42) or by hypoventilation when breathing oxygen in normal subjects (34) In the obstructive group there was a significant correlation between $\overline{P}_{PA}-\overline{P}_{W}$ and Pa_{CO_2} at rest and during exercise. The correlation coefficient was approximately of the same order as between these parameters and Sa_{O_2} (Table IV) Emirgil *et al* (14) in a similar study showed that the correlation between Pa_{CO_2} and both the pulmonary pressure and the pulmonary vascular resistance was always lower than that with Sa_{O_2} The absence of a significant correlation in the restrictive group is due to the absence of *hypercapnia* in these patients.

In the obstructive group Sa_{O_2} and Pa_{CO_2} were correlated at rest ($r = -0.580$ $p < 0.01$) and *during exercise* ($r = -0.658$ $p < 0.001$) This, together with the significant correlation between these variables and $\overline{P}_{PA}-\overline{P}_{W}$ shows that the elevated $\overline{P}_{PA}-\overline{P}_{W}$ is mainly a consequence of deranged gas exchange inherent in these patients.

Several investigators have demonstrated the significance of an increase in hydrogen ion concentration in the genesis of pulmonary hypertension (15 28 36) In this study no significant correlation was found between $\overline{P}_{PA}-\overline{P}_{W}$ or the pulmonary artery mean pressure and pH in either group This might be due to the fact that the change in pH was small in this material The lack of significant correlation however is in accordance with other studies, showing that patients with chronic obstructive lung disease usually maintain a fairly normal pH (2 14) For this reason some investigators did not find a significant correlation between the pulmonary artery pressure and pH in chronic obstructive disease (2) In 8 patients with chronic bronchitis with acute respiratory failure Abraham *et al.* (2) found no significant correlation between pH and pulmonary artery pressure either during the acute illness or recovery

It has been postulated that the reduction in the pulmonary vascular bed plays a minor role in the development of pulmonary hypertension in chronic obstructive lung disease (18 29) This was based on the following findings 1) Removal of 50 % of the vascular bed results in only slight elevation of resting pulmonary artery pressure (10) 2) There was no correlation between the degree of anatomic destruction and right ventricular hypertrophy in patients with emphysema (11) 3) The pulmonary arterial hypertension is reversible (29) However the elevation of $\overline{P}_{PA}-\overline{P}_{W}$ in the restrictive group at rest to the same level as in the obstructive group in the present study in spite of normal blood gases, points to the importance of a restricted vascular bed as a cause of pulmonary hypertension. Simi-

larly the elevation of the pressure gradient during exercise was too high to be caused by the slight hypoxemia present in this group

Harvey *et al* (27 30) studied the pressure flow relationship in terms of diastolic pressure gradient and found an increase in the diastolic pressure gradient with decreased oxygen saturation and increased hydrogen ion concentration. In the present study the correlations between Sa_{O_2} or Pa_{CO_2} and $\overline{P}_{PA}$—$\overline{P}w$ were higher than those between the blood gases and P_{PA_d}—$\overline{P}w$ (Table IV) This might be attributed to the fact that the pulmonary artery mean pressure is more sensitive to changes in blood gases than is the pulmonary artery diastolic pressure and to technical difficulties in measuring the diastolic pressure, particularly in the presence of the ventilatory swings of pressures.

There has been a controversy as to whether the cardiac output in chronic obstructive lung disease is normal high or low (6 17 26 44) It has been demonstrated that cardiac output decreases with age in normal subjects (7 22) and this was suggested as a factor responsible for the disagreement regarding cardiac output in obstructive lung disease (37) This is illustrated in the present study in which the regression lines of the cardiac output on the oxygen uptake were compared with the regression lines in healthy young (13) and elderly subjects (22) Fig 9

The stroke volume was virtually normal at rest in both groups. When both males and females were included, the increase in stroke volum from rest to exercise was 8 % in the obstructive group ($p > 0.05$) which is about 50 % lower than the increase reported in elderly male subjects (22) The increase in the restrictive group was 23 % ($p < 0.01$)

The stroke volume depends on the filling and emptying conditions of the heart. The pressure load to which the right and left hearts were subjected was essentially identical in the two groups. Furthermore the right and left ventricular filling pressures were identical in both groups. Thus the difference in stroke volume is probably to be attributed to the increase in intrathoracic pressure which alters the negative pressure surrounding the heart to zero (atmospheric) or positive pressure (9 38) thereby reducing the effective filling pressure of the central capacity vessels and of the cardiac chambers. Nakhjavan *et al* (41) have demonstrated that, in patients with emphysema with hyperinflation of the lungs and a low diaphragmatic position the blood flow into the thorax was greatly reduced or completely arrested during inspiration. Another contributing factor is that physical inactivity results in small blood volume and instability of the tone of capacity vessels. One or more of these factors may explain the smaller stroke volume during exercise in patients with obstructive disease.

As previously mentioned, the pulmonary artery wedge pressure was in both groups within the r[illegible]
compared to values r[illegible]
elderly subjects, alth[illegible]
are high compared
healthy young

(22) ascribed the high wedge pressure in healthy elderly subjects to the lower distensibility of the myocardium as the stroke volume increased normally during exercise. The low compliance of the myocardium may also explain the high wedge pressure during exercise in the restrictive group which also had a normal increase of stroke volume. In the obstructive group, however the high wedge pressure during exercise was associated with a small stroke volume. This might reflect a left ventricular dysfunction. However in these patients the ECG during exercise did not show any significant signs of myocardial disease although this does not exclude its presence. Some investigators have shown that the left ventricular function was not impaired in chronic lung disease (12, 45). Recently however Baum *et al* (3) demonstrated a left ventricular dysfunction in most of the patients with chronic obstructive lung disease studied by them.

There are few published reports on the effects of obstructive or restrictive ventilatory dysfunction on the central hemodynamics. In the obstructive group a close relationship was recorded between the degree of airway obstruction and the pressure difference across the pulmonary vascular bed ($\bar{P}_{PA}-\bar{P}_W$) but in spite of this no significant effect was found on the cardiac output. Consequently the pulmonary vascular resistance increased with increasing airway obstruction. There was also a close relationship between airway obstruction and the degree of hypoxemia. This is in agreement with other studies (14). Harris *et al* (25) demonstrated an increase in pulmonary vascular resistance by hyperventilation in patients with chronic bronchitis and attributed the increase in pressure gradient and pulmonary vascular resistance to the mechanical effect of hyperventilation in the presence of increased airway resistance. In contrast to their result the blood gases in this study were abnormal while ventilation was only slightly increased. It emerges that the large $\bar{P}_P-\bar{P}_W$ and consequently PVR, could be ascribed either to the effect of blood gases or to the increase in intrathoracic pressure, or most probably to both factors.

Lim and Brownlee (37) and Lockhart *et al* (38) found some evidence that $\bar{P}_W$ increases with increasing intrathoracic pressure in chronic obstructive lung disease. However in contrast to their findings and those of Harris *et al* (25) $\bar{P}_W$ in the present study did not increase with decreasing FEV and the increase in $\bar{P}_P-\bar{P}_W$ was essentially caused by an increase in $\bar{P}_{PA}$. There is a probability that the effect of increased intrathoracic pressure on the wedge pressure in this study was concealed by the counter-effect of the decreased central blood volume.

At rest there was no effect on central hemodynamics with increasing degree of restriction. During exercise it was shown that, at approximately identical levels of cardiac output, the mean pulmonary artery and wedge pressures were lower in patients with marked degree of restriction (b) than in patients with less degree of restriction (a). The difference in age between the two subgroups was too small to account for the difference in pressures. Also both subgroups had

the same degree of hypoxemia, while Pa_{CO_2} was higher in (b) so that the blood gases cannot be responsible for it. It seems likely that the low pressures in patients with marked restriction are probably caused by the effect of high negative intrapleural pressure particularly during inspiration, on the transmural pressure (8). As the effect on the pulmonary artery pressure and wedge pressure during exercise was of the same magnitude, the pressure difference $\overline{P}_{PA}$—$\overline{P}_W$ and pulmonary vascular resistance were virtually unchanged. This finding might explain the observation made by Wade and Bishop (44) that in pulmonary fibrosis the hypoxemia during exercise is accompanied by a relatively mild pulmonary hypertension.

Summary

1. The cardiac output was normal in relation to oxygen uptake at rest and during exercise in both the obstructive (A) and restrictive (B) groups. It was high in one patient in A and during exercise only in one patient in B.
2. The stroke volume increased from rest to exercise by 8 % (p > 0.05) in group A and by 23 % (p < 0.01) in group B.
3. In both groups the mean intracardiac and intravascular pressures at rest were normal except for a slightly elevated $\overline{P}$ [illegible]. During exercise most pressures [illegible] pla [illegible] wide variation from norm [illegible] mark [illegible] lev [illegible] ted. The $\overline{P}_{PA}$ [illegible] and PVR [illegible] also increased [illegible] from [illegible] ed 11 mm Hg at rest and 19 mm Hg during exercise in both groups.
4. $\overline{P}_{PA}$—$\overline{P}_W$ was highly significantly correlated with the degree of arterial desaturation and Pa_{CO_2} increase in group A but respectively less and non-significantly in group B.
5. $\overline{P}_{PA}$—$\overline{P}_W$ and PVR increased with increasing degree of obstruction, but were virtually unchanged with increasing degree of restriction.
6. In neither group was the cardiac output affected by the ventilatory dysfunction.

References

1. Abraham, A.S., Cole, R.B. & Bishop, J.M. Reversal of pulmonary hypertension by prolonged oxygen administration to patients with chronic bronchitis. *Circ. Res.* 23, 147, 1968.
2. Abraham, A.S., Cole, R.B., Green, I.D., Hedworth-Whitty, R.B., Clarke, S.W. & Bishop, J.M. Factors contributing to the reversible pulmonary hypertension of patients with acute respiratory failure studied by serial observations during recovery. *Circ. R.* 24, 51, 1969.
3. Baum, G.L., Schwartz, A., Llamas, R. & Castillo, C. Left ventricular function in chronic obstructive lung disease. *New England J. Med.* 285, 361, 1971.
4. Bevegård, S., Holmgren, A. & Jonsson, B. The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume. *Acta physiol. scand.* 49, 279, 1960.
5. *Bevegård, S.* Studies on the regulation of the circulation in man. With special reference to the stroke volume and the effect of muscular work, body position and artificially induced variations of the heart rate. *Acta physiol. scand.* Suppl. 200, 1962.

6 Blount, S G Cardiac output in pulmonary emphysema I *Pulmonary circulatio* Ed. W Adams and L Veith, Grune and Stratton, New York, 1959
7 Brandfonbrener M Landowne, M. & Shock N W Changes in cardiac output with ge *Circulation 12* 557 1955
8. Burton A.C. The relation between pressure and flow in the pulmonary bed. In *Pulmonary circulation*. Ed W Adams and I Veith, Grune and Stratton, New York, p 26 1959
9 Christie, R.V The elastic properties of the emphysematous lung and their clinical significance. *J Clin Invest. 13* 295 1934
10 Cournand, A., Riley R.L., Himmelstein, A. & Austrian, R Pulmonary circulation and alveolar ventilation-perfusion relationships after pneumonectomy *J Thorac Cardiovasc Surg 19* 3 1950.
11 Cromie J.B. Correlation of anatomic pulmonary emphysema and right ventricular hypertrophy *Amer Rev Resp Dis. 84* 657 1961
12. Davies H. & Overy H R Left ventricular function in cor pulmonale. *Chest 58* 8, 1970
13. Ekelund, L.-G & Holmgren A. Central hemodynamics during exercise *Cir Res. 20 & 21* Suppl. 1 1967
14 Embrgll, C., Sobol B.J Herbert, W.H. & Trout K.W Routine pulmonary function studies as key to the status of the lesser circulation in chronic obstructive pulmonary disease. *Amer J Med 50* 191 1971
15 Enson Y Giuntini C. Lewis, M L Morris, T Q Ferrer M.I & Harvey R.M. The influence of hydrogen ion concentration and hypoxia on the pulmonary circulation. *J Cli Invest 43* 1146 1964.
16 Euler U S. von, & Liljestrand, G Observations on the pulmonary arterial blood pressure in the ca *Act physiol scand 12* 301 1946
17 Evans, T O Van der Reis, L & Selzer A. Circulatory effects of hronic pulmonary emphysema. *Ame Heart J 66* 741 1963
18. Ferrer M I Disturbances in the circulation in patients with cor pulmonale *Bull. N Y A ad M d 41* 942, 1965.
19 Fishman, A.P Fritts, H.W Jr & Cournand, A Effects of acute hypoxia and exercise on the pulmonary circulation. *Circulation 22* 204 1960
20. Fishman, A.P Fritts, H.W J & Cournand, A. Effects of breathing carbon dioxide upon the pulmonary circulation. *Circulation 22* 220 1960.
21 Gabriel, S. The difference between the pulmonary artery diastolic pressure and the pulmonary wedge pressure in chronic lung disease. *Acta med scand. 190* 555 1971
22. Granath A., Jonsson, B. & Strandell, T Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. *Acta med scand 176* 425 1964
23 Granath, A. & Strandell, T Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. *A ta med. and 176* 447 1964
24 Harris, P & Heath, D *The human pulmonary circulation*. E. & S. Livingstone, Edinburg and London, p 279 1962.
25. Harris, P Segel, N Green, I & Housley E The influence of the airways resistance and alveolar pressure on the pulmonary ascular resistance in chronic bronchitis. *Cardiovasc Res. 2* 84 1968
26. Harvey R.M., Ferrer M.I Richards, D W J & Cournand, A. Influence of chronic pulmonary disease on the heart and circulation. *Amer J M d. 10* 719 1951
27 Harvey R.M Enson, Y & Ferrer M.L Further considerations of the causes of pulmonary hypertension in cor pulmonale *Bull Physio-Pathol. esp 3* 623 1967
28. Harvey R.M., Enson, Y Bettl, R., Lewis, M.L., Rochester D F & Ferrer M.I Further observations on the effect of hydrogen ion concentration on the pulmonary circulation. *Circulation 35* 1019 1967
29 Harvey R M The influence of hydrogen ion in the control of pulmonary artery pressures in patients with obstructiv dis-

ease of the lungs. *Med Thorac* 22 108 1965.

30. Harvey R.M Enson, Y & Ferrer M.I.: A reconsideration of the origins of pulmonary hypertension. *Chest* 59 82, 1971

31 Holmgren A., Jonsson, B Levander M., Linderholm, H., Sjöstrand, T & Ström, G Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory asthenia) *Act med and* 158 413 1957

32. Holmgren A., Jonsson, B. & Sjöstrand, T: Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke olume at different work intensities. *Act physiol. scand* 49 343 1960.

33 Jonsson, B. Cardiac catheterization. In *Clinical Physiology* Ed. T Sjöstrand, Scand. University Books, Stockholm, 1967

34 Kaijser L. Limiting factors for aerobic muscle performance *Act physiol. scand* Suppl. 346 1970

35 Kaltman, A.J Herbert, W.H., Conroy R.J & Kossmann, C.E. The gradient in pressure across the pulmonary ascular bed during diastole. *Circulation* 34 377 1966.

36. Liljestrand G Chemical control of the distribution of the pulmonary blood flow *A ta physiol. scand* 44 216 1958.

37 Lim, T.P.K. & Brownlee, W.E. Pulmonary hemodynamics in obstructiv lung disease. *Dis. hest* 53 113 1968.

38 Lockhart A., Tzareva, M., Nader F Le blanc P Schrijen F & Sadoul, P Elevated pulmonary artery wedge pressure t rest and during exercise in chronic bronchitis. *Cli Sci.* 37 503 1969

39 Malmberg R. Gas exchange in pulmonary tuberculosis *S and J p Dis* 47 262, 1 67

40 Motley H.L Cournand, A Werko, L., Himmelstein, A & Dresdale D The influence of short period of nduced acute anoxia upon pulmonary artery pressures in man *Amer J Phy iol.* 150 315 1947

41 Nakhja an F.K Palmer W.H & McGregor M I uence of respira on on enous return in p lmonary e ... ... la-tion 33 8 1966.

42. Rokseth, R.. The effect of altered blood carbon dioxide tension and pH on the human pulmonary circulation. *Sca d J clin. Lab Invest* 18 Suppl. 90 1966

43 Rossier P.H et Bühlmann, A. Cor pulmonale et pathophysiologie alvéolaire. *Cardiologia* (Basel) 5 132, 1954

44 Wade G & Bishop J.M.: *Cardiac output and gional blood flow* Oxford, Blackwell 1962.

45 Williams, J.F J Childress, R.H., Boyd D.L., Higgs, L.M. & Behnk R.H Left ventricular function in patients with chronic obstructive pulmonary disease. *J clin I* st 47 1143 1968.

46 World Health Organization Chronic cor pulmonale. *Wld. Hlth. Org* ec h R p *Ser* 213 1961

CHAPTER VI

DETERMINANTS OF PHYSICAL WORKING CAPACITY IN OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

The study here reported is based on the results presented in Chapters III—V It was demonstrated in patients with obstructive disease that the physical working capacity (W_B) was closely associated with low ventilatory capacity and in restrictive patients was related to the blood volume. It was also demonstrated that the diffusing capacity of the lung was reduced and the pulmonary vascular resistance increased during exercise in the two groups. The purpose of the present study was to determine the relative importance of the measures of lung function, diffusing capacity and some cardiovascular factors, such as the pulmonary vascular resistance and stroke volume for the determination of physical working capacity in patients with obstructive and restrictive lung disease

Results

The data have been reported in the respective chapters, and mainly the results of the correlation study will be mentioned.

Matrix correlation and multiple correlation analysis were performed using 10 variables in the obstructive group (A) and 13 variables in the restrictive group (B) (Tables I—II) In group A the highest correlation was found between W_B and the indices of ventilatory capacity and $D_{L_{CO}}$ ($p < 0.001$) W_B was also significantly correlated with PVR and Pa_{O} ($p < 0.01$) The correlations between W_B and SV Sa_O were only of probable significance ($p < 0.05$)

Using two independent variables together with W_B as a dependent variable in a multiple regression analysis there was no improvement in the correlation when FVC was combined with either FEV or MVV_{40}. The inclusion of $D_{L_{CO}}$ with FEV in the regression analysis increased the correlation coefficient from 0.819 to 0.914 When $D_{L_{CO}}$ was combined with MVV_{40}, the correlation coefficient increased from 0.858 to 0.942 and the standard error of estimate decreased from 106 to 71

The regression equations for the above relationships are written

$W_B = 14.56 + 5.2\ MVV_{40} + 13.11\ D_{L_{CO}}$ SE 71 r = 0.942

$W_B = 27.67 + 154.63\ FEV + 15.98\ D_{L_{CO}}$ SE 86 r = 0.914

When SV was combined with either FEV_1 or MVV there was no improve

Table I Correlation matrix in group A

	W	IRC	FEV	MVV	Dl_{CO}	Pa_{O_2}	PVR	$\bar{P}_{PA}-\bar{P}_W$	SV	Sa_{O_2}
W	1.000 (24)									
IVC	0.830 (1)	1.000 (24)								
FEV	0.823 (24)	0.878 (24)	1.000 (21)							
MVV	0.818 (24)	0.911 (24)	0.898	1.000 (4)						
Dl_{CO}	0.010 (21)	0.649 (21)	0.619 (21)	0.623 (1)	1.000 (21)					
Pa_{O_2}	0.562 (22)	0.603 (22)	0.591 (22)	0.576 (22)	0.675 (1)	1.000 (22)				
PVR	−0.575 (22)	−0.496 (22)	−0.506 (22)	−0.409 (22)	−0.544 (21)	−0.572 (22)	1.000 (22)			
$\bar{P}_{PA}-\bar{P}_W$	0.103 (22)	−0.181 (22)	−0.411 (22)	−0.445 (22)	−0.487 (21)	−0.659 (22)	0.906 (22)	1.000 (22)		
SV	0.196 (22)	0.311 (22)	0.257 (22)	0.553 (22)	0.410 (21)	0.189 (22)	−0.467 (22)	−0.266 (22)	1.000 (22)	
Sa_{O_2}	0.131 (22)	0.306 (22)	0.432 (22)	0.161 (22)	0.442 (21)	0.841 (22)	−0.534 (22)	−0.680 (22)	0.241 (22)	1.000 (22)

< 0.422 not significant
$0.533 > r > 0.422$
$0.65 > r > 0.535$
> 0.632

$n = 22$

number of observations in parentheses

Table II Correlation matrix in group B

	W_B	TLC	VC	FEV	MVV_{40}	D_{LCO}	Pa_{O_2}	Sa_{O_2}	SV	PVR	THb	BV	HV
W_B	1.000 (17)												
TLC	0.182 (17)	1.000 (17)											
VC	0.394 (17)	0.812 (17)	1.000 (17)										
FEV_1	0.348 (17)	0.685 (17)	0.868 (17)	1.000 (17)									
MVV_{40}	0.380 (17)	0.621 (17)	0.777 (17)	0.918 (17)	1.000 (17)								
D_{LCO}	0.624 (16)	0.107 (16)	0.130 (16)	0.204 (16)	0.137 (16)	1.000 (16)							
Pa_{O_2}	−0.004 (16)	0.466 (16)	0.376 (16)	0.346 (16)	0.219 (16)	−0.023 (16)	1.000 (16)						
Sa_{O_2}	−0.012 (15)	0.320 (15)	0.218 (15)	0.306 (15)	0.210 (15)	0.338 (15)	0.500 (15)	1.000 (15)					
SV	0.536 (15)	0.376 (15)	0.3 4 (15)	0.251 (15)	0.142 (15)	0.610 (15)	−0.248 (15)	0.161 (14)	1.000 (15)				
PVR	−0 18 (15)	0.172 (15)	0.179 (15)	0.039 (15)	−0.033 (15)	−0.277 (15)	0.190 (15)	0.611 (14)	0.095 (15)	1.000 (15)			
THb	0. 04 (1)	0.390 (17)	0.431 (17)	0.388 (17)	0.375 (17)	0.686 (16)	0.103 16)	0.017 (15)	0.550 (15)	0.012 (15)	1.000 (17)		
BV	0.517 (17)	0.577 (17)	0.537 (17)	0.419 (17)	0.411 (1)	0.351 16)	0.11 16	0.164 (15)	0.50 (15)	0.1 (15)	0.78 (17)	1.000 (17)	
HV	0.367 (16)	0.140 (16)	0.243 (16)	0.207 (16)	0.1 (16)	0.184 (16)	0.006 (16)	0.103 (14)	0.390 (15)	0.386 (15)	0.5 4 (10)	0.419 (16)	1.000 (16)

< 0.530 not significant
0.620 > r > 0.530
0.776 > > 0.620
> 0.776

n = 17

number of observations in parentheses

reduction of diffusing capacity In previous studies it has been shown that the diffusing capacity and the pulmonary capillary blood volume were reduced in patients with pulmonary fibrosis (9) The argument cited above is supported by the positive correlation between W_B, THb $D_{L_{CO}}$ and SV

Summary

This study showed that in obstructive patients the low physical working capacity was associated with low ventilatory and diffusing capacities. The latter were found to be most suitable when combined for the assessment of physical working capacity The coexistence of cardiac dysfunction plays a minor role in the limitation of physical performance. In restrictive patients the low physical working capacity was attributed to the small circulatory dimensions and reduced diffusing capacity

References

1 Ghnntni, C & Marani, M. Capacitance of pulmonary asculature in hronic pulmonary disease. *S and J esp Dis* Suppl 77 66 1971

Holmgren, A Experience of measuring th diffusing capacity d ring exercise with the steady stat method of Filley modified by Linderholm. I *Palm nary diffusing ca pacity on ercise* Ed. M Scherrer H Huber Publishers, 1971

3 Jones, N.L., Jones, G & Edwards, R.H T Exercise tolerance in chronic airway obstruction. *Amer ev Re p Dis 103* 477 1971

4 Linderholm, H Diffusing capacity of the lungs as a limiting factor for physical work ing capacity *Acta m d ad 163* 61 1959

5 Marcus, J H M Lean, R L Duffell G.M. & Ingram, R H Exercise performance in relation to the pathophysiologic type of hronic obstructive pulmonary disease *Amer J M d 49* 14 1970

6. Rankin, J McNeill, R.S. & Forster R.E. The effect of anemia on the alveolar-capillary exchange of carbon monoxide in man. *J Clin. I vest 40* 1323 1961

Simonsson, B G Malmberg R. & Berglund, E.: Pulmonary gas exchange t rest and during exercise in chronic bronchitis. *S and J esp Dis. 50* 245 1969

8. Sjöstrand, T *Clinical Physiology* Scand. University Books, Svenska Bokförlaget Stockholm, 1967 *p* 526.

9 Y P N *P lmonary blood vol me health and di ase* Chapter 15 p. 48, Lea & Febiger Philadelphia 1969

CHAPTER VII

GENERAL DISCUSSION

I Pathophysiological changes

Two groups of patients were studied A) 24 patients with obstructive lung disease and B) 17 patients with restrictive lung disease.

The two groups were similar with regard to their anthropometric data and circulatory dimensions (THb BV and HV) (Chapter II)

Physical working capacity

The physical working capacity (Chapters III and VI) measured as W_B was in the obstructive group 52 % of the normal value predicted from the equation given by Irnell and Linder (27) and in the restrictive group 55 %. The cause of interruption of the exercise test in the two groups was dyspnea in about 90 % of cases. Other causes, such as fatigue in the legs, general fatigue and ectopic beats, were recorded for some patients in both groups, either alone or associated with dyspnea. In the *obstructive group* there was a highly significant correlation between the physical working capacity (W_B) on the one hand and the ventilatory capacity and diffusing capacity on the other The circulatory dimensions and the central circulation were of minor importance for the working capacity compared with the ventilatory or diffusing capacities. This is shown by the weak correlation between W_B and the circulatory dimensions and the data from the measurements of the central circulation.

The present results are in agreement with those from previous studies in obstructive disease, which showed a high relationship between the maximal physical performance, on the one hand, and ventilatory capacity (26 29 34 43) and diffusing capacity (34) on the other

The working capacity in the *restrictive group* in contrast to the obstructive group, was related neither to lung volumes nor to ventilatory capacity The significant finding that W_B was highly correlated with the ventilatory capacity in the obstructive group but not in the restrictive group although the ventilatory capacity was practically of the same order in the two groups, suggests that the physical working capacity is not limited by the volume ventilated per se but by other factors associated with airway obstruction. Levison and Cherniack (31) suggested that the oxygen cost of increased ventilation during exercise may be an important factor limiting the work performance in patients with obstructive lung disease. Freedman (18) ascribed the inability to sustain 15 sec MVV for a longer period to the fatigue of the respiratory muscles. The low physical working capacity in the restrictive group was not related to the ventilatory capacity but to the reduced diffusing capacity and to the small circulatory dimensions, presumably caused by the effect of the disease on physical activity

The physical working capacity (W_{70})

in the *obstructive group* was related to circulatory factors and partially to the effect of ventilatory dysfunction. Physical inactivity results in small dimensions of the circulation. The increase in intra thoracic pressure might influence the filling pressures of the heart and results in a small stroke volume. In the *restrictive group* however restrictive ventilation seemed not to have a direct effect on W_{170}, while circulatory dimensions were important factors. In patients with restricted ventilation due to severe idiopathic scoliosis the low W_{170} was ascribed mainly to the reduction in circulatory dimensions rather than to the direct effect of ventilatory limitation (19) In both groups W_{170} was slightly correlated with $D_{L_{CO}}$ ($p < 0.05$)

The physical working capacity (W_R) was found to be significantly higher in sitting than in supine position in the two groups. This might be ascribed in both groups mainly to the high airway resistance and work of breathing in recumbent position (10 36 37) The working capacity (W_{max}) was also found to be higher in sitting than in supine position in normal elderly subjects (45) while the reverse was found in patients with sarcoidosis who had marked orthostatism during exercise (46)

The respiratory rate at submaximal work load showed wide variations and was not related to the degree of airway obstruction in the obstructive group, but a weak negative correlation was found between respiratory rate at 200 kpm/min and the vital capacity in the restrictive group It has been claimed that disturbed lung function may be suspected if the respiratory rate increases to more than 35 breaths/min (33) This seemed probably true in restrictive disease, where high respiratory rates were found in patients with small lung volume.

Ventilation, gas exchange and diffusing capacity

There is a general agreement that arterial hypoxemia in obstructive disease is caused mainly by uneven ventilation/perfusion (28, 35 43) Although the reports on restrictive disease (pulmonary fibrosis) agree that hypoxemia becomes more accentuated during exercise yet they are conflicting as regards its mechanism (39 44 48) The present investigation aimed to elucidate this problem (Chapter IV)

During exercise the alveolar ventilation was more adequate in the restrictive than the obstructive group, as evidenced by the blood gases and by the regression equation of V_A on V_{O_2} The V_D, V_D/V_T V_A/Q (calculated) and veno-arterial shunt demonstrated a more pronounced inequality of ventilation/perfusion in the obstructive than in the restrictive group The diffusing capacity was slightly reduced, but identical in the two groups. The $P(A—a)_{O_2}$ was markedly increased in both groups, at rest to a greater extent in the obstructive than in the restrictive group while during exercise $P(A—a)_{O_2}$ was unchanged in the obstructive but increased in the restrictive group In 11/24 patients in group A and 5/16 patients in group B $P(A—a)_{O_2}$ during exercise was unchanged or decreased.

Piiper (38) in his theory of unequal distribution of diffusing capacity has

demonstrated that when the diffusing capacity is unequally distributed in relation to perfusion the $P(A-a)_O$ would be greater than in a lung with the same diffusing capacity and perfusion but with equal distribution. Applying this concept to the present material the mean ratios $V_A/\dot{Q}$ and $D_{L_{CO}}/Q$ were determined in the two groups. As Q was not measured at the same time as V_A and $D_{L_{CO}}$, it was calculated by means of the regression equation of the cardiac output on the oxygen uptake obtained during the hemodynamic study (Chapter V). The results showed that during exercise $V_A/\dot{Q}$ was lower in group A than in group B while the reverse applied to $D_{L_{CO}}/\dot{Q}$. Thus, the observed changes in $P(A-a)_O$ and consequently the arterial hypoxemia, were mainly ascribed to uneven ventilation/perfusion in the obstructive group and uneven diffusion/perfusion in the restrictive group.

Central hemodynamics

The effects of hypoxemia on the central hemodynamics of patients with chronic lung disease have been extensively studied by many investigators, particularly in obstructive lung disease. The importance of hypoxemia lies in its intimate interrelationship with pulmonary hypertension. In the present material the oxygen saturation at rest was slightly reduced in the obstructive group but normal in the restrictive group, and during exercise both groups showed a mild degree of hypoxemia. The present investigation showed in the two groups, at rest and during exercise a normal regression of pressures on the cardiac output when compared with pressures reported in healthy elderly subjects (21, 22) except for the regression of $\overline{P}_{PA}$ in both groups and RV in group B both of which were slightly significantly above normal. The latter showed a tendency to above-normality in group A. At rest $\overline{P}_{PA}$ averaged 20 mm Hg in both groups compared to 16 mm Hg in normal material ($p < 0.05$). During exercise most pressures displayed a wide individual variation from normal to highly elevated. It is important to point out that the average age in the control material was 70 years compared to 58 years in group A and 54 years in group B so that the pressures in the patient material might probably be higher than in normal subjects of comparable age. The elevation of pulmonary artery pressure was studied in terms of pressure difference between the pulmonary artery mean pressure and the mean pulmonary wedge pressure $\overline{P}_{PA}-\overline{P}_W$ as an increase of wedge pressure contributes to the elevation of pulmonary artery pressure (20, 24). In the two groups the increase in $\overline{P}_W$ during exercise contributed to about 50 % of the increase in pulmonary artery mean pressure. In the obstructive group $\overline{P}_{PA}-\overline{P}_W$ was significantly related to oxygen unsaturation and to increase of carbon dioxide tension. This is in agreement with previous studies, where hypoxemia has been considered the dominating factor in the genesis of pulmonary hypertension (1, 12, 13, 14, 16, 23). Also hypercapnia has been considered an important factor (12, 17, 23, 41). In the restrictive group $\overline{P}_{PA}-\overline{P}_W$ was identical with that in the

obstructive group in spite of the normal arterial blood gases at rest and less marked oxygen unsaturation during exercise. This was ascribed to the effect of reduction of the vascular bed. The present study did not demonstrate any relationship between $\overline{P}_{PA}$—$\overline{P}_{W}$ and arterial pH in the two groups. The reports in the literature are conflicting as regards this relationship (cf 1 12, 13 24 41)

The controversy in published reports as to whether cardiac output is normal high or low in obstructive lung disease (4 14 49) has been ascribed to the effect of age on the cardiac output (32) The present investigation showed in both groups almost normal cardiac output in relation to oxygen uptake both at rest and during exercise when the effect of age (5 45) was eliminated.

The present findings are in agreement with those of other investigators who reported normal or almost normal cardiac output at rest and in response to exercise in obstructive lung disease (30 49) Similarly the cardiac output was reported to be normal in patients with pulmonary fibrosis (49) Recently Tammivaara — Hilty (47) reported in patients with severe obstructive lung disease low cardiac output compared to the same normal material as used in the present study However several of her patients had left ventricular impairment and respiratory insufficiency Several authors have reported different patterns of cardiovascular dysfunction in obstructive lung disease, e.g PP and BB patients (15) bronchial and emphysematous type (7) low output and hypoxemic pattern (8) The different patterns of abnormality seemed to be due to the difference in arterial blood gases and to an anatomical destruction of the vascular bed in individual patients, and the difference in published reports was explained by the difference in selection of patients (8) The obstructive group in the present material included a mixed type of bronchitic and emphysematous patients, and the cardiac output was within ±2 SD i.e. it varied between high normal to low normal. However the causes of this interindividual variation in cardiac output were not analysed in the present study

The stroke volume increased from rest to exercise by 8 % p >0 05 in the obstructive group and by 23 % p <0 01 in the restrictive group without significant difference between the two groups. Figures of 13 and 16 % have been reported as normal values in healthy young and elderly subjects respectively (11 22) The small increase of stroke volume from rest to exercise in the obstructive group was ascribed mainly to the effect of increased intrathoracic pressure on the filling conditions of the heart.

Effects of obstructive and restrictive ventilatory dysfunction

The effects of obstructive ventilatory dysfunction on the hemodynamics were studied (Chapter V) $\overline{P}_{PA}$—$\overline{P}_{W}$ and consequently PVR, were found to increase with increasing degree of airway obstruction, but no effect on the cardiac output was noted. This was mainly caused by the effect of hypoxemia and probably by the increase in intrathoracic pressure.

With increasing degree of restriction there was no effect at rest on the pres-

sures, cardiac output or oxygen saturation. During exercise, on the other hand, it was demonstrated that patients with marked degree of restriction (VC <50 % of predicted) had $\bar{P}_{PA}$ and $\bar{P}_{W}$ which were about 7 mm Hg lower than patients with lesser degree of restriction (VC >50 % of predicted) but $\bar{P}_{PA}-\bar{P}_{W}$ and PVR were practically identical (Table VI Chapter V) This difference in pressures could not be satisfactorily explained by the difference in age, blood flow blood volume or blood gases, but was presumably due to a decreased negative intrapleural pressure level.

II. Selection of function tests

1 *Physical performance*

In patients with *obstructive disease* the physical working capacity expressed as the work load performed at the breaking point of exercise (W_B) was found to be more advantageous than the work load at heart rate 130/min (W_{130}) or the heart rate at the exercise breaking point (HR_B) W_B was found to reflect the degree of severity of disease as evidenced by the high correlation with the indices of ventilatory capacity ($p < 0.001$) Moreover it is always measurable. The disadvantage of using W_{130} was that the work could not be increased to this heart rate in all patients also an approximate circulatory steady state could not be achieved in some patients. It was weakly correlated to both ventilatory capacity and circulatory dimensions. HR_B, similarly to W_B, was significantly correlated with the ventilatory capacity but the level of significance was not as high as with W_B. Moreover HR_B might be influenced by a high resting heart rate which was recorded in several patients in both groups.

In patients with *restrictive disease* neither of the three parameters W_B, W_{130} and HR_B was significantly correlated to the lung volumes or ventilatory capacity The only correlations found were with circulatory dimensions.

It is suggested therefore, that in patients with obstructive disease W_B is the parameter of choice for assessment of the influence of the respiratory disease on physical working capacity In restrictive patients, on the other hand, W_B does not reflect the ventilatory impairment.

In patients with lung disease the work test should be performed in sitting position, as both types of patients had a higher work capacity in sitting than in supine position. This is in contrast to other conditions, e.g in some inactive cardiac patients before chest surgery (25) and in sarcoidosis (46) in whom orthostatism is marked during exercise and consequently the work pulse is higher in supine than in sitting position.

In the present study W_B was measured as the highest load the patient was able to sustain for 6 minutes. In many instances the patient had to interrupt the work earlier and W_B had to be recalculated (see methods, Chapter I) It may be profitable, therefore, to use exercise periods of shorter duration, e.g. 2—3 minutes, or to use progressive exercise tests (29) A comparison between these methods would be of value.

2. *Ventilatory capacity*

Spirometry is a simple and one of the most widely used function tests which

provide valuable information as to whether the patient has an obstructive or restrictive ventilatory dysfunction. The relative importance of the total lung capacity and its subdivisions and of the indices of ventilatory capacity for the assessment of physical performance was deduced from the regression analysis of W_B on these variables (Chapter III)

In the *obstructive group* the indices of ventilatory capacity FVC, FEV_1 MVV_{40} and MVV_{free}, were highly significantly correlated with W_B ($p < 0.001$) These parameters were more important than the lung volumes or RV/TLC % and FRC/TLC % The present studies (Chapters III and VI) showed that, by determining W_B and FEV_1 or MVV_{40}, it is possible to decide whether or not the low physical working capacity in a particular patient is due to impairment of the ventilatory function. The ventilatory capacity in obstructive patients was also found to influence other respiratory functions such as $D_{L_{CO}}$ arterial oxygen saturation and the pressure difference across the pulmonary vascular bed.

In the *restrictive group* spirometric data were not correlated with the physical working capacity In contrast to the obstructive group the functional derangements in this group had generally no causal relationship to restrictive ventilatory dysfunction. Accordingly in this material and within the present range of restriction, the ventilatory tests gave an anatomical measure of restriction but did not reflect a functional derangement.

3 *Test of diffusing capacity*

The measurement of diffusing capacity is necessary for the assessment of function in certain lung diseases. However the different methods and techniques used are the cause of wide disparity in the results reported (9)

In the obstructive group the steady state exercise $D_{L_{CO}}$ was found to be reduced in 9 of 21 patients. Low values of $D_{L_{CO}}$ were found in patients with low FEV_1 or MVV_{40} ($p < 0.01$) and high V_D/V_T ratio ($p < 0.001$) which might indirectly signify that $D_{L_{CO}}$ is to a large extent affected by the degree of uneven ventilation. However in the present material emphysema was a dominating disease, so that the reduction in $D_{L_{CO}}$ also reflected a reduction in the available surface area for diffusion. For practical purposes it is difficult, however to quantify the reduction caused by each factor

Cadigan *et al.* (9) have shown that the low values of D_L steady state compared to D_L single breath in patients with emphysema were due to uneven ventilation and that the patients with severest emphysema had the lowest steady state $D_{L_{CO}}$ Briscoe *et al.* (6) have shown that in emphysema the V_A/Q ratio averaged 2 24 in well ventilated alveoli and 0 23 in poorly ventilated alveoli. The corresponding end-capillary oxygen saturation was 97.5 % and 76 % They concluded that inhomogeneity of this degree invalidates the calculation of diffusing capacity based on a single mean alveolar CO tension. These factors have caused some authors to condemn the use of the steady state method in obstructive lung disease (3 6 9) The findings in the present investigation suggest, as in earlier reports (2) that in obstructive disease

$D_{L_{CO}}$ steady state is to be looked upon as a test for overall estimation of the oxygen transfer rather than a measure of the diffusing capacity

Thus the measurement of $D_{L_{CO}}$ when combined with FEV_1 or MVV_{40}, was found to increase the precision of prediction of the physical working capacity in these patients (Chapter VI)

In the restrictive group $D_{L_{CO}}$ was reduced in 9 of 16 patients. Although the reduction of $D_{L_{CO}}$ in the restrictive group could explain the marked fall of Pa_{O_2} in some patients, yet a marked fall in Pa_{O_2} was observed also in the presence of normal $D_{L_{CO}}$. It seemed from the present investigation that in restrictive disease the Pa_{O_2} level is more influenced by abnormal regional distribution of diffusion in relation to perfusion than by the impairment of the oxygen transfer across the alveolar capillary membrane per se. The presence of high dead-space and veno-arterial shunt, together with the reduced $D_{L_{CO}}$, in restrictive patients adds to the complexity of the interpretation of diffusing capacity

For the reasons given above, and because the procedure is relatively disagreable and cannot be applied to severely disabled patients, the measurement of $D_{L_{CO}}$ by the present method seems to be of little value as a routine test. However it is a sensitive test for the assessment of lung function in special circumstances and measures one aspect of lung function that cannot be measured by other methods.

4 *Test of cardio-circulatory function*
Heart catheterization was not discriminatory as regards the pressure flow relationship. In both the obstructive and restrictive groups the pressures were virtually identical at rest and during exercise. The increase of the pressure gradient across the vascular bed and of the pulmonary vascular resistance was approximately of the same magnitude in the two groups. The regression of cardiac output on oxygen uptake did not differ significantly in the two groups (cf regression equations in Chapter V)

One of the main values of performing heart catheterization in patients with lung disease is to determine whether or not a patient has a normal pulmonary vascular resistance. Being an elaborate investigation, it is therefore important to determine which type of patient is to be catheterized. In obstructive patients the pulmonary vascular resistance was related to airway obstruction. Considering the upper limit of the pulmonary vascular resistance index in our laboratory to be 3 units, it was found that in 10 patients with FEV % lower than 50 % of predicted (subgroup III Table V Chapter V) the mean pulmonary vascular resistance index was increased both at rest and during exercise, while in patients with FEV % between 50—75 % of predicted (subgroup II) the pulmonary vascular resistance was increased during exercise only It is suggested, therefore, that heart catheterization may be restricted to patients with FEV % lower than 50 % of predicted.

CHAPTER VIII

GENERAL SUMMARY AND CONCLUSIONS

Forty-one patients with chronic lung disease selected among patients referred to this department for functional evaluation were studied. The material was functionally classified into two groups: A) 16 men and 8 women, mean age 58 years, with obstructive lung disease and B) 12 men and 5 women, mean age 54 years, with restrictive lung disease.

The two groups were similar with regard to age, anthropometric measurements and circulatory dimensions (THb, BV, HV). They had reduced but practically the same, ventilatory capacity. The physical working capacity at the breaking point of exercise (W_B), measured as the highest load the patient was able to sustain for 6 minutes, was about 50 % of normal.

The low physical working capacity (W_B) was associated with low ventilatory and diffusing capacities in the obstructive group, while in the restrictive group it was associated with small circulatory dimensions and reduced diffusing capacity. Probably these were the main factors limiting work performance in these patients. The central circulation was a minor factor in the limitation of physical performance in the two groups.

The pulmonary gas exchange data in the obstructive group demonstrated a normal average Pa_{CO_2} at rest and somewhat elevated during exercise. The arterial P_{O_2} was slightly lower than normal at rest but, in most patients, did not fall during exercise. In the restrictive group Pa_{CO_2} was somewhat lower than in the obstructive group both at rest and during exercise, and the Pa_{O_2} was somewhat higher at rest but fell markedly in some patients during exercise. On the basis of the findings of V_D, V_D/V_T, venoarterial shunt and measured diffusing capacity it was suggested that the arterial hypoxemia is due mainly to uneven ventilation/perfusion in the obstructive group and to uneven diffusion/perfusion in the restrictive group.

The hemodynamic studies demonstrated in the two groups almost normal cardiac output at rest and in response to exercise. The mean pressures at rest were normal except for a slightly elevated pulmonary artery pressure. During exercise most of the pressures showed a wide variation from normal to markedly elevated. The pulmonary vascular resistance was increased in both groups. The wide pressure difference across the pulmonary vascular bed $\overline{P}_{PA}—\overline{P}_{W}$ was related to the oxygen unsaturation and Pa_{CO_2} increase in the obstructive group but less so and non-significantly respectively in the restrictive group. It was not related to the pH in either group.

The stroke volume increased from rest to exercise by 8 % in the obstructive group and 23 % in the restrictive group. The small increase in the obstructive group was ascribed mainly to factors associated with increased intrathoracic pressure.

The pressure difference across the pulmonary vascular bed and the pulmonary vascular resistance increased with increasing airway obstruction but were virtually unchanged with increasing degree of restriction. It seemed from the present investigation that pulmonary hypertension, and consequently cor pulmonale, are consequences of obstructive ventilatory dysfunction. In restrictive patients pulmonary hypertension was also present but was not related to the degree of restrictive ventilatory impairment, and hence other factors might be of importance for the development of cor pulmonale.

It is apparent from the results of the present investigations that alterations in respiratory function have a causal relationship to obstructive ventilatory dysfunction but not to restrictive ventilation In restrictive disease functional derangements seemed to have a weak relationship to the cause of restriction.

For the evaluation of physical working capacity in obstructive disease, W_B was found to be the measure of choice for assessment of the influence of respiratory disease on physical performance In restrictive disease W_B was not related to the ventilatory impairment.

The diffusing capacity and heart catheterization were found in the present study to have a low practical discriminatory value in obstructive and restrictive disease

Suggested investigation procedure

As screening tests for evaluation of lung function, the exercise tolerance test (W_B) and the test of ventilatory capacity are suggested in the first place. When these tests display a deviation from ordinary values of about 30 % or more, the following investigations are recommended

A) In obstructive patients measurement of arterial oxygen and carbon dioxide tensions at rest and during exercise.

B) In restrictive patients measurement of arterial blood gases as in A and, if P_{aO_2} displays an abnormal reduction during exercise, determination of diffusing capacity and blood volume are recommended.

References

1 Abraham, A.S. Cole R.B. & Bishop J.M. Reversal of pulmonary hypertension by prolonged oxygen administration to patients with chronic bronchitis. *Circ Res.* *3*, 147 1968.

2. Bates, D.V. Knott, J.M.S. and Christie R.V. Respiratory function in emphysema in relation to prognosis. *Quart J M d 25* 137 1956.

3 Bjure, J & Söderholm, B. Pulmonary diffusing capacity critical review of its value as lung function test. *S and. J clin. Lab Invest 22* 167 1968.

4. Blount, S.G. *Cardiac output in pulmonary emphysema.* I *Pulmonary ci culation.* Ed. W Adams and L Veith, Grune and Stratton, New York, 1959

5. Brandfonbrener M., Landowne, M. & Shock, N.W. Changes in cardiac output with age. *Circulation. 12* 557 1955

6. Briscoe, W.A Cree, E.M., Filler J Houssay H.E.J and Cournand A. Lung volume alveolar entilation and perfusion interrelationships in chronic pulmonary emphysema. *J ppl. Physol. 15* 785 1960

7 Burrows, B Fletcher C.M., Heard, B.E., et al The emphysematous and bronchial types of chronic airways obstruction clinicopathological study of patients in London and Chicago. *Lancet* 1 830 1966.

8. Burrows, B Kettel L.J Niden, A.H., t al. Patterns of cardiovascula dysfunction in chronic obstructv lung disease. *New England J M d. 286* 912, 1972.

9 Cadigan, J.B Marks, A., Ellicott, M.F Jones, R.H., and Gaensler E.A. An analysis of factors affecting the measurement of pulmonary diffusing capacity by the single breath method. *J Clin. I vest.* 40, 1495 1961

10. Dubois, A.B Resistance to breathing. *Handbook of Physiology* section 3 ol. 1 p 451 American Physiological Society Washington, 1964

11 Eklund, L.-G & Holmgren, A. Central hemodynamics during exercise. *Cir R s.* *20 & 21* Suppl. 1 1967

12. Emirgil, C., Sobol, B.J Herbert, W.H. & Trout, K.W.. Routine pulmonary function st... t... status of the lesser ... obstructive pulmo- n... *M d 50* 191 1971

13 E... Lewis, M.L Mor ... & Harvey R.M. T... en ion concentra t... pulmonary circu ... 1146 1964

14 E... cs, L. & Selzer A... onic pulmonary ... *66* 741 1963

15 ... J Reeves, J.T ... bronchopul- ... n transport in ... t ... *J Med.* 44 26, 1...

16 F... H.W Jr & Cournand A Effects of acu hypoxia and ex... on the pulmonary circulation. *Cir... 2* 301 1960

17 Fri... n, A.P Fritts, H.W J & Cournand, A Effects of breathing carbon dioxide pon the pulmonary circulation. *Circula ion 22* 270 1960

18. Freedman, S Sustained maximum voluntary entilation *Resp Physiol.* 8 230 1970

19 Freyschuss, U Nilsonne U Lundgren, K.D Idiopathic scoliosis in old ge. *Acta med d 192* 41 1972.

20 Gabriel, S The difference between the pulmonary artery diastolic pressure and the pulmonary wedge pressure in chronic lung disease *Acta med scand 190* 555, 1971

22. Granath, A., Jonsson, B & Strandell, T Circulation in healthy old men studied by right heart catheterization t rest nd during exercise in supine and sitting position. *Act med. scand. 176* 425 1964

21 Granath, A. & Strandell, T Relationships between cardiac output, stroke volume and intracardiac pressures t rest and during exercise in supine position and some anthropometric data in healthy old men. *Acta med scand 176* 447 1964

23 Harvey R.M., Ferrer M.I Richards, D W J & Cournand, A. Influence of chronic pulmonary disease on the heart and circulation. *Amer J Med. 10* 719 1951

24 Harvey R.M., Enson, Y & Ferrer M.I : Further considerations of the causes of pulmonary hypertension in cor pulmonale. *Bull Physio-Pathol. resp. 3* 623 1967

25 Holmgren A. Choice of information according to purpose (abstract) *Scand J esp Dis* Suppl. 77 1971 p. 136

26. Irnell, L. A study of bronchial asthma. *Act med. scand* suppl 419 1964

27 Irnell, L. & Linder Unpublished observations. Quoted in ref no. 26 p 27

28 Jones, N.L. Pulmonary gas exchange during exercise in patients with chronic i w y obstruction. *Clin. Sci. 31* 39 1966.

29 Jones, N.L., Jones, G & Edwards, R H.T Exercise tolerance in chronic airway obstruction. *Amer ev Resp Dis 103* 477 1971

30 Kitchin, A.H Lowther C.P and Matthews, M.B. The effects of exercise and of breathing oxygen-enriched air on the pulmonary circulation in emphysema. *Clin. Sci., 21* 93 1961

31 Levison, H. & Cherniack, R.M. Ventilatory cost of exercise in chronic obstructive pulmonary disease. *J ppl Physol. 25* 21 1968

32. Lim, T.P.K. & Brownlee, W.E Pulmonary hemodynamics in obstructive lung disease *Dis hest 53* 113 1968.

33 Linderholm, H. *F actional capacity nd exercise toleranc in patients with impair d lu g f ction. Clinical cardiopulmonary physiology* Grune & Stratton Inc., New York, 1960

34 Marcus, J.H M Lean, R L., Duffell, G M. & Ingram, R H. Exercise performance in relation to the pathophysiologic type of chronic obstructive pulmonary disease. *Amer J M d 49* 14 1970

35 Mellemgaard, K. The alveolar-arterial oxygen difference in chronic obstructive lung disease. *S and J esp Dis 48* 23 1967

36 Moreno F & Lyons, H.A Effect of body posture on lung volumes. *J ppl. Physol 16* 27 1961

37 Petit, J.M., Milic Emili G and Sadoul P L'influence de la position corporelle sur l travail entilatoire dynamique pendant l exercice musculaire chez l individu sain. Arch. Internat. physiol. Bioch. 68 437 1960

38. Piiper J Unequal distribution of pulmonary diffusing capacity and the alveolar arterial P_{O_2} differences theory *J ppl. Physiol 16* 493 1961

39 Read J & Williams, R.S Pulmonary ventilation. Blood flow relationships in interstitial disease of th l ngs. *Amer J M d 27* 545 1959

40 Rodman, T & Sterling F.H *P lmonary emphysema d elated l g dis ses* V C. Mosby Comp. p 3 1969

41 Rokseth, R. The effect of altered blood carbon dioxide tension and pH on the human pulmonary circulation. *Sca d J lin Lab I st 18* Suppl. 90 1966

42 Shaw D.B., Grover R F Reeves, J T and Blou t G J Pulmonary circulation in chronic bronchitis and emphysema *Brit Heart J* 27 674 1965

43 Simonsson, B G Malmberg R. & Berglund, F Pulmonary gas exchange t rest and during exercise in chronic bronchitis. *S and. J esp Dis 50* 245 1969

44 Staněk V Wid maký J Kasalický J Na rátil M Daum, S & Levinský L. The pulmonary gas exchange during exercise in patients with pulmonary fibrosis. *Scand J p Dis 48* 11 1967

45 Strandell, T Circulatory studies on healthy old men with special reference t the limitation of th maximal physical working capacity *Acta med scand* suppl 414 1964

46. Svanborg N Studies on the cardiopulmonary f nction in sarcoidosis *Act med scand* suppl 366 1961

47 Tammivaara Hilty R. Oxygen transport and physical working capacity in severe chronic obstructiv lung disease *Act Universitatis Upsaliensis* 1972

48. Vale, J R. Effect of exercise and O breathing gas exchange in hronic bronchitis and in diffuse, restricti pulmonary disease. *Scand J resp Dis.* 51 316 1970

49 Wade, G & Bishop, J.M. *C dia output and regional blood flow* Oxford, Blackwell, 1962.

ACKNOWLEDGEMENTS

I wish to express my gratitude to all persons at the Departments of Clinical Physiology Pulmonary Diseases and Medicine at Karolinska sjukhuset and at the Central Dispensary in Norrtälje who have contributed to the present investigations.

I should like to thank in particular

Professor Torgny Sjöstrand, the Head of the Department, for his continuous encouragement and valuable advice, and for all facilities he placed at my disposal.

Associate Professor Alf Holmgren, Head of Department of Clinical Physiology at the Thoracic Clinics, for his constructive criticism and stimulating discussions

My colleagues in this Department for their technical assistance, valuable discussions and suggestions.

Miss Margareta Eriksson, Miss Inga Jarmar Miss Asta Johansson and Miss Ulla Oswaldsson for all kinds of technical assistance during the investigations.

Mrs Birgitta Edholm for excellent assistance in the preparation of this manuscript.

Mr John Hogg for the correction of the manuscript.

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases and Karolinska Institute.

Acta Medica Scandinavica

Supplementum 547

Factors Related to Sudden Death in Acute Ischaemic Heart Disease

A community study in Helsinki

By Matti Romo

Distributed by The Almqvist & Wiksell Periodical Company, Stockholm, Sweden

Acta Medica Scandinavica

Supplementum 547

Factors Related to Sudden Death in Acute Ischaemic Heart Disease

A community study in Helsinki

By Matti Romo

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica.*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes.

/ *Subscription Rates*

Per annum = two volumes.

In Denmark, Finland, Iceland, Norway Sweden and the Netherlands Sw kr 170, incl. postage.

Other countries. Sw kr 200, incl. postage.

Chief Editor

Professor Jan G. Waldenström, MD
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Box 1712
S-111 87 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksell Informationsindustri AB
S-751 81 Uppsala, Sweden

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 547

FROM THE RESEARCH DEPARTMENT OF THE FINNISH HEART ASSOCIATION AND FROM THE FIRST DEPARTMENT OF MEDICINE, UNIVERSITY CENTRAL HOSPITAL, HELSINKI, FINLAND

FACTORS RELATED TO SUDDEN DEATH IN ACUTE ISCHAEMIC HEART DISEASE

A COMMUNITY STUDY IN HELSINKI

by

MATTI ROMO

HELSINKI 1972

Vammala 1972 Vammalan Kirjapaino Oy

ACKNOWLEDGEMENTS

This study is based on material collected by the Ischaemic Heart Disease Register in Helsinki. It is part of an international study co-ordinated by the World Health Organization. The present study was carried out during the years 1969—1972 at the Research Department of the Finnish Heart Association and at the First Department of Medicine, Helsinki University Central Hospital.

I wish to express my deep gratitude to my former chief and teacher Professor Pentti I. Halonen, M.D Head of the First Department of Medicine, Helsinki University Central Hospital, and of the Wihuri Research Institute for the valuable advice he offered, and for the encouragement he gave.

The subject of this study was suggested by Docent Pentti Siltanen, M.D Head of the Cardiovascular Laboratory First Department of Medicine, Helsinki University Central Hospital, and of the Research Department of the Finnish Heart Association, whose initiative led to the establishment of the IHD Register in Helsinki. My most sincere thanks are due to him for the valuable aid he gave me at all stages of my work.

I am also greatly indebted to Associate Professor Kalevi Pyörälä, M.D for his valuable advice and constructive criticism at the manuscript stage

From Docent Gottfried Härtel, M.D I received much valuable advice regarding the manuscript of this work, for which I am indebted.

Mr Seppo Sarna, Lic phil., was responsible for the computer analysis, and the multiple discriminant analysis was performed in co-operation with him. Mr Pyry Matti Vasama, M.Sc., performed the statistical analyses. Mr Uljas Attila, M.Sc., designed and executed most of the illustrations. He also read my English manuscript, which was finally revised by Mr Peter Jones, B.A. Mr Einari Liukkela drew part of the illustrations. Miss Irmeli Roti, Hum. Cand., checked the references. To all these persons I am deeply grateful.

Furthermore, I am grateful for help received from the personnel of the IHD Register from the interviewer nurses, Mrs. Kyllikki Miettinen, Tuula Liinanto and Sirkka Laakso, and from the Secretary Mrs. Marita Puranen.

An indispensable prerequisite for the activities of the IHD Register was co-operation with numerous hospitals, institutes and authorities. It is not possible to mention all those who have given their help in the course of this work, but I wish to express my thanks to them all.

The establishment of the IHD Register was made possible by a grant from the National Pensions Institute. Its activity was also supported by the Finnish Heart Association and the City of Helsinki. The personal grants to the Author from the Finnish Heart Association and the Paavo Ilmari Ahvenainen Foundation are gratefully acknowledged.

Finally I wish to thank my wife Sirkka, who also drew some of the illustrations, for her patience and support during the time in which I was engaged in this work.

Helsinki, October 1972

Matti Romo

CONTENTS

I INTRODUCTION AND AIM OF THE STUDY

Sudden coronary death (SCD) is without doubt one of the most difficult problems in contemporary medicine. The establishment of coronary care units in the 1960's has contributed a great deal to our understanding of its mechanism. In recent years, mobile coronary care units (MCCU) have drawn ever earlier phases of the acute ischaemic heart disease (AIHD) into the limelight. The essential role of primary arrhythmias was thus pointed out. It has also been found that most of these early arrhythmias can be succesfully treated or even prevented if the treatment is started early enough.

However this progress benefits only a minority of all patients with acute ischaemic heart disease at present. It is a fact that two thirds of the total mortality rate in AIHD still consists of deaths outside hospitals. It is natural that the pre-hospitalization phase of acute attacks has attracted increasing interest.

Those who succumb to sudden coronary death in their most active years, often quite unexpectedly comprise a large group among the victims concerned. However it can be noted on closer consideration that many of these have previously suffered from symptomatic atherosclerotic diseases or that in them symptoms have occurred prior to the attack, which simply have not been recognized as being of serious nature.

During the last two decades, the literature of the subject dealing with the risk factors of ischaemic heart disease (IHD) has increased to a really extravagant degree. The same risk factors which are predisposive to all manifestations of IHD in general seem to be associated with sudden coronary death, although their predictive power may differ in different manifestations of the disease. The factors expressly predisposive to sudden death are only imperfectly known as yet. Additional information is also needed as regards premonitory symptoms and those events which immediately precede sudden fatalities, as well as the circumstances in which they occur Full understanding of all these aspects is a prerequisite for any programme of prevention or control.

The vital statistics of various countries, together with international comparative studies, have clearly indicated that great differences exist in the occurrence of arteriosclerosis, and of various manifestations of IHD as well as in IHD mortality It follows that the experiences pertaining to any one country are not as such applicable to another country In world vital statistics Finland is conspicuous in that it has the highest IHD mortality of males of all countries or regions. Finnish females also range high, although not highest of all. During the most recent years the mortality of IHD has continuously increased in Finland, as it has done in many other developed countries. This alarming trend can hardly be reversed unless attention is more strongly centred on what takes place outside hospitals.

In 1969 an Ischaemic Heart Disease Register was established in Helsinki in co-operation with the World Health Organization (WHO). An attempt has since been under taken to register all cases of AIHD con cerning persons aged 65 years or younger within the population of Helsinki.

The Ischaemic Heart Disease Register furnished an opportunity for studying the occurrence of sudden coronary death within an entire community with its total mortality and morbidity of AIHD as a background. It was possible to compare the characteristics of those dying suddenly to those whose death was more delayed and to those who survived their AIHD attack.

The aim of the study was

1 To determine the incidence of sudden coronary death in Helsinki and to gain a quantitative understanding of its importance.

2. To study demographic characteristics, previous diseases and other risk factors in three comparison groups sudden coronary deaths (SCD) non-sudden coronary deaths (NSCD) and survivors (SURV) and to assess the possibilities of identifying in advance those persons who are exposed to the highest risk of dying suddenly

3 To study the prodromal symptoms with regard to pointing out those alarming symptoms, if any which might indicate a mandatory need of intervention.

4 To study the circumstances in which the sudden fatalities occurred in order to reveal potential immediately predisposing factors.

II REVIEW OF THE LITERATURE

The literature concerning various aspects of sudden coronary death has escalated to a large extent. Experimental and clinical stud ies have served attempts at clarifying the electrophysiological events concerned and their pathological basis in sudden fatalities. Epidemiological studies, on the other hand, have furnished information on the natural history of sudden coronary death in certain communities.

The first part of the present review is dedicated to the definition of sudden coronary death. The pathological basis of sudden coronary death and its mechanism are also briefly considered. Some prominent epidemiological studies concerning sudden coronary death in different countries are reviewed. Ultimately certain characteristics, previous diseases and other risk factors related to sudden fatalities are discussed.

1 DEFINITION OF SUDDEN CORONARY DEATH

A major difficulty in comparing and inter preting the results of previous studies on sudden death has been lack of a uniformly accepted definition (Kuller 1966) Nearly all studies exclude violent death, such as suicide, accidental death and poisoning However there is disagreement as to what should be the duration of the fatal attack and as regards ts unexpected nature, which has also been used as a criterion by many authors.

In 1959 a WHO expert committee found that at the time no sufficient evidence existed to justify the use of sudden death as an index for coronary heart disease. It was stated, however that it could become one if more information were gathered on its occurrence in an unselected population and on its various causes, such as pulmonary embolism, cerebral accidents and dissecting aneurysm on the side of coronary heart disease (Hypertension and coronary heart disease 1959) The committee suggested that the term sudden death should be employed to mean a death occurring instantaneously or within a few minutes.

In 1969 an expert committee supported by the Scientific Council on Arteriosclerosis and Ischaemic Heart Disease of the Inter national Society of Cardiology and by the Councils on Arteriosclerosis and Epidemi ology of the American Heart Association suggested the following definition. Sudden unexpected death is a death occurring in stantaneously or within an estimated 24 hours of the onset of acute symptoms and signs» The suggestion also included a proposal that sudden deaths should be further subdivided into those occurring within one hour 1—12 hours and 12—24 hours, respec tively

In spite of these recommendations, the time interval used in various studies differs from 15 minutes to 24 hours and even longer In some studies no time limit whatsoever is applied instead, the place of death or the unexpected nature of the death have served as a criterion for selection. Deaths in hospitals or other institutions are often excluded from studies concerning sudden death. The practice varies as regards unwitnessed deaths with unknown duration of the fatal attack.

Other more serious difficulties arise when

it is desired to define the concept of sudden coronary death. Even if a definite time limit were specified for the fatal attack and the unexpectedness of the event were precisely defined, the difficulty remains that, even in autopsy it is often difficult to demonstrate the cause of sudden death with certainty. There are no generally accepted criteria for autopsy findings which might justify entry of AIHD as a cause of death. *The pathologist can, however rule out other* reasons for sudden death and furnish indirect evidence for AIHD as being the most probable cause of death. It is further noted that autopsy is infrequent in many communities. In many population studies having a low autopsy rate sudden deaths have been attributed to AIHD if no other obvious cause of death can be pointed to

2. ISCHAEMIC HEART DISEASE AS A CAUSE OF SUDDEN DEATH

The references to different causes of sudden death in numerous text-books on forensic medicine are mainly based on medico-legal autopsy series. These series may be biased but, all the same, the essential role played by IHD as a cause of sudden death becomes clearly evident in nearly all autopsy studies. Diseases of heart and aorta are considered responsible for 45 to 85 per cent of sudden deaths. The largest contribution in this group is that of IHD varying from 50 to 79 per cent of the group mentioned (Kuller 1966 Uotila 1970)

In most series of medico-legal autopsies no differentiation is made with regard to duration of the terminal event. Such studies exist, however which suggest that the role of IHD as a cause of sudden death becomes even more prominent when sudden death is defined in terms of a comparatively short time interval. In a study by Spain et al. (1960), the material concerning 1329 consecutive autopsies was divided into three categories according to the duration of the fatal episode less than one hour from one to three hours and undetermined time (un witnessed death). The authors showed that in the first category 91 per cent of the deaths of males, but only 52 per cent of those of females, were due to IHD In the second category the proportion of IHD was less than 55 per cent, and in the third category 61 per cent in males and only 35 per cent *in females. Similar results were noted* in a cross-sectional study of sudden death in Baltimore (Kuller et al. 1967). In the age group of 40 to 64 years 91 per cent of the deaths of white males occurring within two hours were due to IHD or hypertensive heart disease the corresponding figure for white females was 95 per cent. In contrast, only 69 per cent of white males' and 59 per cent of white females' deaths occurring between 2 and 24 hours after the onset of symptoms were due to these diseases. In the group of unwitnessed deaths the figures were 79 and 64 per cent, respectively

The figures stated above are in good agreement with the current understanding of the mechanism of sudden coronary death. Cardiac arrest causes death within a few minutes, while the duration is somewhat longer with other mechanisms of natural death. Unwitnessed deaths constitute a heterogeneous group of cases, with variable duration of the fatal event and probably also differing in other respects from the group of the witnessed deaths.

3 MECHANISM OF SUDDEN CORONARY DEATH

One of the major benefits from coronary care units has been that better clarification of the mechanism of sudden coronary death has been attained and the great importance of the early arrhythmias has been revealed.

Hellerstein and Turell (1964) reported that ventricular asystole was the commonest

terminal electrocardiographic finding in a series of hospitalized patients who succumbed to sudden death. However most investigators have shown that ventricular fibrillation is more common (Lown et al. 1967 Cross 1968, Adgey et al. 1969 Dixon 1970). This is also in agreement with animal experiments (Caroll et al. 1965).

Many reports, e.g from Belfast, have pointed out the high frequency of rhythm disturbances at the very beginning of an acute attack. Patients arriving at hospital have very often already passed the most dangerous phase of their acute illness and the rhythm disturbance pattern has also changed. In a recent report from Belfast (Adgey et al. 1971), based on patients who were reached by a mobile coronary care unit (MCCU) within one hour of the onset of an AIHD attack, no less than 233 of 284 patients (82 per cent) had some kind of arrhythmia, and in 170 patients dysrhythmia appeared within the first hour and required therapy before the patient was considered fit for transport to hospital. Brady cardia was highly common during the first hour (in 31 per cent) and it has been shown as enhancing the ectopic ventricular activity (Han 1969). Hypoxia with all the consequences attendant upon it, together with the electrical instability of the myocardial tissue, creates conditions conducive to ventricular fibrillation. Primary ventricular fibrillation occurred in 5.5 per cent of patients admitted to a coronary care unit within 4 hours after the onset of symptoms, as contrasted with an incidence of 0.4 per cent when admission was delayed (Lawrie et al. 1968). According to Pantridge and Geddes (1967) ventricular fibrillation is 25 times more frequent during the initial 4 hours than during the remaining part of the first 24 hours.

Many authors have reviewed the topic (Surawicz and Pellegrino 1964, Lovell and Prineas 1971, Lown and Wolf 1971 James 1972).

4. PATHO-ANATOMICAL FINDINGS

The morphological changes in myocardial infarction are not visible by conventional methods until some six hours have passed after onset of the process. It is, therefore, natural that if sudden death is defined as one occurring within one hour these changes are usually absent. It is peculiar that in most studies a certain proportion of cases displayed pathological changes, suggesting a myocardial infarction. In the series of sudden coronary deaths reported the frequency of fresh myocardial infarction has usually varied from 8 to 24 per cent (Adelson and Hoffman 1961 Franco 1962, Spiekerman et al. 1962, Hansen 1968 Luke and Helpern 1968, Wikland 1971), but even a higher frequency (47) is recently reported (Scott and Briggs 1972).

In many cases of sudden death attributed to IHD the sole finding is diffuse coronary atherosclerosis, often more advanced than could be expected on the basis of age. Patches of myocardial fibrosis and scars after an old infarction are also common, and the number of scars usually exceeds that of the clinically recognized episodes (Sievers 1963, Wikland 1971)

The frequency of fresh coronary thrombosis varies in different studies from 15 to 69 per cent (Croce et al. 1960, Crawford et al. 1961 Franco 1962, Hallerman 1962, Mitchell and Schwartz 1965 Hansen 1968, Kagan et al. 1968, Luke and Helpern 1968, Spain et al. 1969 Rissanen 1970 Titus et al. 1970 Scott and Briggs 1972). Many reasons have been speculatively suggested for this variability including differences in autopsy technique and differences between the populations studied and in definitions of sudden death. On the whole, however it can be said that the occurrence of a fresh thrombus in the coronary arteries seems to be rarer in cases of sudden death than in cases with a more delayed yet eventually fatal course. Spain and Bradess (1960 and 1970) claim that the

frequency of thrombosis correlates with the patient's survival period, increasing with a longer duration of the attack. They also dispute the common idea that myocardial infarction is preceded by thrombotic occlusion on the contrary they suggest that thrombosis may be a sequela of myocardial infarction. This contention has received emphatic support from Baroldi (1965). Meadows (1965) and Kagan et al. (1968), too, have failed to find evidence in favour of the traditional idea that an occlusion of a main coronary artery has to occur before myocardial infarction develops.

Spain et al. (1963) also stress the importance of collateral circulation, and they claim that lack of such circulation may be an essential cause of sudden death. In contrast, Baroldi (1969) also demonstrated an extensive collateral network in patients who had died suddenly without any preceding symptoms indicative of coronary heart disease. He states that occlusion of coronary arteries is of less importance in the development of myocardial infarction, which originates in the cells of the myocardium.

James has emphasized the need for careful studies of the small coronary arteries (1967), the blood supply of the sinus node (1968) and the histology of the conduction system (1969). Lesions of the conduction tissue in cases of sudden death have also been reported by Lumb and Shacklett (1962).

It can be stated that, at present, it is often impossible in autopsy to demonstrate by conventional technique the cause of sudden death with certainty (Edwards 1971 Schwartz and Walsh 1971). The high prevalence of coronary atherosclerosis in our adult population further impedes the determination of the cause of sudden death. Microscopical examination improves the accuracy of diagnosis to a certain degree and the same is acchieved by more recent histochemical and other methods (The Pathological Diagnosis of Acute Ischaemic Heart Disease 1970). Their value and usefulness in practice remains to be demonstrated.

5 POPULATION STUDIES

Apart from autopsy studies, several cross-sectional or retrospective as well as prospective studies performed both in U.S.A. and in Europe have aimed at determining the frequency of sudden coronary death in a given specific population.

U.S.A.

Spiekerman et al. (1962) studied all deaths in Rochester Minnesota, a small community with a population of 30 000 having an exceptionally high autopsy rate in the United States during the five-year study period, 1947 to 195.. There were altogether 1026 deaths at the age of 20 years or older of which 691 (6" per cent) were autopsied. Of these patients 221 had died of IHD and 114 of these deaths (52 per cent) were sudden. No definition of sudden death was given.

The Health Insurance Plan (H.I.P.) of Greater New York Study comprised 55 000 men aged 25—64 years. During a four year period beginning in 1961 881 men experienced their first myocardial infarction or sudden death. Sudden death, defined in the study as one occurring within 24 hours, accounted for 271 (30.6 per cent) of these first events (Shapiro et al. 1965 Weinblatt et al. 1968).

An extensive community study of sudden death was carried out in Baltimore in 1964—1965 (Kuller et al. 1966 a, b c, 1967 Kuller 1969). Sudden death was defined as a death occurring within 24 hours and concerning a person not restricted to his home, to a hospital or to another institution and not unable to function in the community over more than 24 hours prior to death. 60 per cent of all IHD deaths occurred within 24 hours and 61.4 per cent of all sudden deaths were due to AIHD

Several other community studies have revealed closely similar figures as those mentioned above (Zukel et al. 1959 Eisenberg et al. 1961 Bainton and Peterson 1963).

In addition to the community studies mentioned, there are also several prospective epidemiological studies, many of them with a large cohort and a long follow-up period, but even so these are hampered by a relatively small number of sudden deaths.

Perhaps the most notorious of them all is the Framingham Study in which results from a 12 and 14 year follow-up period have been reported (Kannel et al. 1966 Gordon and Kannel 1971). In a cohort of 5209 persons, 120 died of IHD during 14 years, and 66 of these deaths (55 per cent) occurred within one hour

In the Tecumseh Study 98 deaths due to IHD were noted during the six year period from 1959 to 1965 and 45 of them (46 per cent) occurred within one hour (Chiang et al. 1970).

Some preliminary data are available from the national co-operative pooling project of the U.S.A., which combines the results of seven extensive prospective cardiovascular studies (Stamler 1971). Of 7594 men aged 30—59 and free of IHD at entry 501 experienced a first event of AIHD during the ten-year observation period. 123 (24.6 per cent) of these events were sudden deaths, defined as a death occurring within three hours from the onset of illness.

The studies mentioned above as well as several other prospective studies (Doyle et al. 1959 Pell and d'Alonzo 1964, Stamler 1967), have altogether consistently indicated that sudden death occurs, as the first manifestation of IHD in 20—25 per cent of cases.

Europe

Studies reported from England and Northern Ireland have produced closely similar results to comparable studies in the U.S.A.

Fry studied in his own practice, in London, the incidence of myocardial infarction and sudden death over a period of 17 years, from 1949 to 1966 In a population of 5500 persons on the average, he found 350 new cases of IHD and 149 of them terminated fatally 97 deaths occurred within 24 hours and 80 deaths within one hour (54.4 per cent of all AIHD deaths) (Fry and Dillane 1967).

In Stratford-on-Avon, McWhinney (1968) studied the same problem in an extensive group practice. 28 of 131 events (21.5 per cent) observed over a five-year period were sudden deaths.

All deaths attributed to IHD were studied in one year 1965—1966 in Belfast, Northern Ireland (McNeilly 1967 McNeilly and Pemberton 1968). Various time intervals, including that from onset of symptoms to death, were carefully investigated. Altogether 998 deaths due to IHD were recorded. 332 of them (33 per cent) occurred within the first hour In 127 cases the time interval was not known.

In the Edinburgh Community Study in 1967—1968 all cases of AIHD in people under 70 years were registered (Armstrong 1968, Fulton et al. 1969 Armstrong et al. 1972). This study has been a model for the ischaemic heart disease registers in other countries. The proportion of sudden deaths of all IHD deaths closely parallelled the figures from Belfast. In one year altogether 1298 cases of AIHD were registered, and 42 per cent of them died during the first four weeks. Of these deaths 35.4 per cent occurred within one hour and 58.2 per cent within 24 hours. Deaths within one hour constituted 14.8 per cent of all AIHD cases.

Some data exist concerning IHD deaths outside hospitals in the Scandinavian countries, too.

In Denmark an attempt was made to elicit the morbidity and mortality of myocardial infarction over two months throughout the whole country (Mosbech and Dreyer 1965) The study was based on notified cases from the hospitals' departments of medicine and

on death certificates covering the same two-month period. Altogether there were 1530 deaths in IHD of which 872 (57 per cent) occurred outside hospital.

In Gothenburg Sweden, the local Ischaemic Heart Disease Register started a pre-pilot study in November 1968. So far only results of this pre-pilot study of a three month duration have been published (Fodor 1969). During this period 470 cases of AIHD were registered in all, 166 of them concerning persons under 66 years. 63 of these 166 patients died, 36 outside hospital (i.e. 22 per cent of all AIHD cases and 57 per cent of all AIHD deaths)

Recently Wikland (1971) has studied all medically unattended deaths in Stockholm from IHD over a one-year period. This material of unattended deaths consists of 967 deaths which occurred outside hospitals or other institutions for the aged or chronically ill. 278 of the subjects were under the age of 65 Incidence rates for different age and sex groups are given. Attempts were also made to determine the duration of the fatal attack. This could be done in 595 cases. In 413 cases (69 7 per cent of fatal attacks with known duration) the death occurred within one hour The prevalence of various previous diseases is presented. An analysis of different causes of sudden death other than IHD was made concerning the same period.

Finland

Information about IHD mortality in Finland is available from the official vital statistics, but data concerning sudden coronary death are scanty

In Espoo, a neighbouring town of Helsinki, Gorbatow et al. (1969) investigated all cases of myocardial infarction in 1965 The study was based on hospital records and death certificates. In a population of 77 341 164 events altogether were found during the study year 111 in males and 53 in females. 87 persons died (53 per cent of registered cases) 72 of them (83 per cent of all deaths) outside hospital.

A prospective epidemiological study has been in progress in two different areas in Finland since 1959 as part of an international co-operative study undertaken in seven countries (Karvonen et al. 1970 Keys 1970) A cohort of 1711 men aged 40 to 59 years and free of IHD at entry have been under observation for ten years. 101 IHD deaths were noted. The duration of the fatal attack was known in 88 cases it was less than one hour in 45 cases (51 6 per cent of fatal attacks with known duration) (Punsar 1971)

Several studies seem to suggest that the IHD mortality and hospital admission rate for AIHD have been increasing in Helsinki (Uotila 1945 Varpela 1960 Gorbatow 1961 Sipilä 1966 Tenhu et al. 1970 a, b Uotila 1970 Gorbatow et al. 1971).

In an extensive autopsy series, consisting of all medico-legal autopsies performed at the Department of Forensic Medicine of the University of Helsinki 1923—1942, Uotila (1945) reports that diseases of the heart and aorta were responsible for 59.6 per cent of all these deaths, while IHD constituted 62.9 per cent of these diseases. The corresponding figures in material from the years 1966—1967 of the same department were 85 and 79 respectively (Uotila 1970).

During the years 1959—1968 altogether 3044 cases of AIHD in which death occurred within 24 hours were autopsied at the same department. In this series, deaths within one hour constituted 64 per cent of male and 55 per cent of female cases. 17 per cent of the males and 30 per cent of the females were found dead and the accurate duration of the fatal attack could not be defined. In only 19 per cent of the males and 21 per cent of the females death occurred during the remaining 23 hours (Tenhu et al. 1970 a).

6 FACTORS RELATED TO SUDDEN CORONARY DEATH

During the two most recent decades the literature dealing with the risk factors of IHD has expanded to be voluminous and many extensive reviews have been presented (Epstein 1965 Stamler 1967 Simborg 1970).

Marital status

Studies concerning the possible relationship of marital status to IHD have yielded contradictory results. Most of them have perhaps tended to suggest a certain over-mortality among non-married, in particular widowed persons (Kraus and Lilienfeld 1959 Zalokar 1960 Sheps 1961 Bainton and Peterson 1963, Rees and Lutkins 1967 Parkes et al. 1969). In Belfast, the IHD mortality in the age groups between 40 and 69 years was significantly higher for widowed than for married or for single persons (McNeilly 1967) No relationship between marital status and duration of the fatal attack could be found, however

There are also several studies which have failed to suggest any association between marital status and IHD mortality they include the prospective studies of Framingham (Kannel et al. 1967 a) and Tecumseh (Chiang et al. 1970) as well as the H.I.P study in New York (Shapiro et al. 1969) and the study of Yater et al. (1948)

Social class

The association between social class and IHD has been an object of lively investigation during recent decades (Marks 1967 Antanovsky 1968 Hinkle et al. 1968). Earlier reports seemed to show quite consistently higher incidence of IHD in the higher social classes. In recent years the picture has become rather more obscure, and several reports state an inverse correlation.

Data on the relationship between social class and sudden coronary death are scarce. No correlation was found in Belfast by McNeilly (1967) between social class and occupation on one hand and the duration of the fatal attack of AIHD on the other

In Finland, Sipilä (1966) noted with regard to hospital patients with myocardial infarction in Helsinki that the distribution of social classes parallelled that of the population on the whole. In Espoo, Gorbatow and coworkers found a higher morbidity and mortality of IHD in the lower social classes (Gorbatow et al. 1969). In a study of the IHD mortality in Helsinki, based on death certificates, Gorbatow et al. (1971) observed the highest mortality in the lowest social class, in which the increase in mortality after 1955 was also most striking

Previous ischaemic heart disease

The most indisputable predictor of ultimate death and sudden death from IHD is the symptomatic IHD itself. Several studies have demonstrated the continuously high mortality following a myocardial infarction. Approximately 20 per cent of middle-aged men who survive an initial attack of myocardial infarction die during the following five years (Weinblatt et al. 1968 Stamler et al. 1969), a substantial proportion of them suddenly The mortality is highest during the first six months after the attack, but it remains 5 to 10 times that of the general population for several years (Hagström et al. 1967 Weinblatt et al. 1968 Stamler et al. 1969 Zukel et al. 1969 Chiang et al. 1970, Stamler 1971). The mortality in recurrent attacks is higher than that in initial attacks, although the mode of death in recurrent attacks seems to be delayed rather than sudden (Achor et al. 1956, Weiss 1956 Liebow and Badger 1963, Hagström et al. 1967 Badger and Liebow 1968 Rissanen et al. 1971).

The elevated mortality of patients with angina pectoris is also shown in several studies (Selm 1960, Frank 1968, Weinblatt

et al. 1968, Chiang et al. 1970 Kannel and Feinleib 1970 and 1972, Gordon and Kannel 1971) Angina pectoris combined with hyper tension or with an abnormal electrocardiogram is of particular importance in increasing the fatal risk (Seim 1960 Frank 1968 Oxman et al. 1970 Punsar 1972) In Seim s series of patients with angina pectoris, 37 per cent of IHD deaths were sudden, a figure which, however is rather lower than the frequencies of sudden death usually reported in an unselected population.

Diabetes mellitus

The atherogenic effect of diabetes mellitus is well known (e.g Bradley and Partamian 1965 Robertson and Strong 1968) Evidence of IHD has been demonstrated in half of diabetics over the age of 40 (Bryfogle and Bradley 1957) Premature IHD even at a relatively young age is not infrequent in diabetics.

On the other hand, a significantly elevated prevalence of diabetes mellitus or abnormal glucose tolerance has been demonstrated in patients with various manifestations of IHD (Ostrander et al. 1965 Epstein 1969) An outstanding feature of diabetic myocardial infarction patients seems to be their rather common lack of chest pain as an initial symptom (Bradley and Schonfeld 1962).

In the Framingham as well as the Tecumseh study diabetes mellitus seems to be associated in particular with death in AIHD (Kannel et al. 1967 a, Epstein and Ostrander 1971).

Hypertension

The relationship of elevated blood pressure and IHD is well established (Deming 1968) As an atherogenic factor hypertension increases the incidence of all manifestations of IHD and very distinctly its mortality (Robertson and Strong 1968 Weinblatt et al. 1968 Oberman et al. 1969) Therefore its frequency among those who die suddenly is also naturally high. This has been reported in e.g the Framingham (Kannel et al. 1971) and Tecumseh (Chiang et al. 1970) studies. In fact, the figures from the Framingham study seem to indicate that hypertension has a stronger association with the incidence of sudden death than with any other manifestation of IHD (Dawber and Thomas 1971).

On the other hand, in Baltimore the prevalence of hypertension was higher among those whose death was not sudden compared to those who died suddenly (Kuller et al. 1966 a). Neither could Wikland (1971) in Stockholm find any relation between the history of hypertension and the duration of the fatal attack.

Obesity

Contradictory results have been reported concerning the relationship of obesity and incidence of IHD (Seltzer 1969) A positive correlation was demonstrated e.g by Master et al. (1953) Master and Jaffe (1955) Doyle et al. (1959), Marks (1960), Stamler et al. (1960) Shapiro et al. (1969). On the other hand, e.g Yater et al. (1948), Keys (1954), Sanders (1959) Paul et al. (1963) and Pell and D Alonzo (1964) found no distinct correlation.

More recently prospective epidemiological studies have revealed that obesity like many other risk factors, bears different relations to different manifestations of IHD The Framingham study has shown that obesity adds to the risk of angina pectoris and of sudden death, but not to the incidence of myocardial infarction (Kannel et al. 1967 a and b) Those with a relative weight of more than 20 per cent above the median had a marked excess risk of sudden death. Later on, data from the Tecumseh study have confirmed the association of obesity with SCD (Chiang et al. 1970) On the other hand, no excess prevalence of overweight was found in three autopsy series of sudden

coronary death (Croce et al. 1960 Adelson 1961 Hansen 1968) No association between obesity and autopsy finding of coronary atherosclerosis appeared in the International Arteriosclerosis Project (Montenegro and Solberg 1968)

Smoking habit

Numerous epidemiological studies have quite consistently reported a correlation between smoking habit and incidence of IHD and the exceptions are few (Zukel et al. 1959 Doyle et al. 1962, Bainton and Peterson 1963 Paul et al. 1963 Doyle et al. 1964, Mulcahy and Hickey 1966 Kannel et al. 1967 a, Shapiro et al. 1969 Punsar and Pyörälä 1970) Cigarette smoking seems to be associated with an increased incidence of myocardial infarction and of sudden coronary death, while the connection with angina pectoris has remained uncertain. The risk correlates with the number of cigarettes smoked daily but hardly with the duration of the smoking habit. Pipe and cigar smokers run a relatively small risk, which is close to that of non-smokers. Discontinuation of cigarette smoking results in decrease of both morbidity and mortality

The effect of smoking on the development of IHD is most prominently seen in the young age groups, and it looses much of its strength after the age of 55 years. Many studies, though not all, suggest that cigarette smoking promotes atherosclerosis of the coronary arteries (Auerbach et al. 1965 Strong et al. 1966 Rissanen et al. 1972) as well as other arteries (Auerbach et al. 1968, Sackett et al. 1968)

The mechanism by which smoking exerts its effect is not yet understood though several possibilities exist (Oram 1968 Kjeldsen 1969). It seems likely that, in addition to its potential atherogenic effect, smoking has other more immediate and so far unknown, modes of action. It has been observed that in dogs exposed to cigarette smoke the treshold for ventricular fibrillation becomes subnormal for periods of up to one hour (Webb et al. 1968).

The experience gained in the Framingham study suggests a very distinct relationship between smoking and sudden coronary death (Kannel et al. 1966 1967 a) This association is most clear-cut in heavy smokers, who have a fivefold excess risk of dying suddenly compared to non-smokers. Sudden death was also a common occurrence among smokers in the autopsy series of Spain et al. (1969) and of Spain and Bradess (1970) as well as in an autopsy series of the Ischaemic Heart Disease Register in Helsinki during the pre-pilot period 1969 (Rissanen et al. 1971) Contradictory results have been reported from the Tecumseh study where those who died suddenly had smoking habits equal to those of the population at large (Chiang et al. 1970).

Physical activity

Since the classical work of Morris et al. (1953) was published, the association of physical activity and IHD has been the object of numerous epidemiological studies (Brown et al. 1957 Chapman et al. 1957 Zukel et al. 1959 Brunner and Manelis 1960 Stamler et al. 1960, Paul et al. 1963, McDonough et al. 1965, Frank et al. 1966, Kannel 1967) In some respects, the results have been contradictory although most of them seem to show an inverse correlation between the level of physical activity and the incidence of IHD At least two outstanding studies in the U.S.A., the H.I.P Study in New York and the Framingham Study have indicated that the association of physical activity with the occurrence of sudden death is most strongly in evidence (Shapiro et al. 1969 Dawber and Thomas 1971).

Most of the previous studies are based on occupational physical activity only How ever Frank et al. (1966), Kannel (1967) and

Tibblin (1970) among others, have shown the importance of *off* job activity on the side of on-job activity

While most studies have suggested an association between the level of physical activity and the incidence of myocardial infarction and mortality in IHD its correlation with angina pectoris has remained dubious.

It is not understood how exercise may be able to guard against myocardial infarction and coronary death. In animal experiments, an increase of collateral circulation has been shown (Eckstein 1957). In a large autopsy series, Morris and Crawford (1958) reported fewer fibrous patches and myocardial scars and also slightly fewer occlusions of the coronary arteries among those with active occupations compared to those engaged in sedentary occupations.

Other risk factors

Nearly all prospective epidemiological studies have indicated a strong correlation between the level of serum cholesterol and the incidence of all manifestations of IHD This becomes clearly evident e.g in the Framingham study (Kannel et al. 1966 and 1969). Relationship of IHD with serum triglycerides has also been shown (Albrink and Man 1959 Brown et al. 1965), although this association seems to be weaker than that concerning cholesterol.

A familial aggregation of IHD is shown in many studies (Epstein 1964, Rose 1964, Slack and Evans 1966, Oscherwitz et al. 1968) and the significance of genetic factors both independently and through several confirmed risk factors seems to be established.

Several studies have suggested the importance of the personality pattern and of various psychological characteristics for the development of IHD (Friedman and Rosenman 1960 Friedman 1964 Ostfeld et al 1964 Rosenman et al. 1964, Keith 1966 Rosenman et al. 1967 b Wolf 1969)

Rahe and Lind (1971) in Stockholm gathered data of the changes in life of 39 patients, from a time period covering three years before their sudden death. A significant increase in the subjects' life change intensities during the final six months was noted compared with chronologically equivalent time periods 2 and 3 years earlier So far unpublished data from a study in Helsinki, using the same methodology have come to similar results (Rahe et al. 1972)

Prospective studies have suggested the importance of certain electrocardiographic changes in predicting the group at highest risk to die suddenly Definite evidence of left ventricular hypertrophy carried a very high risk in the Framingham study similar to that of patients who have survived a myocardial infarction (Kannel et al. 1969 Kannel et al. 1970 Gordon and Kannel 1971)

Conduction defects, bundle branch block, and certain disturbances of rhythm, especially ventricular premature beats, have all been demonstrated to constitute an excess risk of dying suddenly (Chiang et al. 1969 Lown et al. 1969 b, Lovell and Prineas 1971 Lown and Wolf 1971)

Numerous other risk factors, which are more or less mutually connected, have been suggested.

A report from Canada has suggested that the hardness of household water might be a factor predisposing in particular to sudden death (Anderson et al. 1969) Results on the same line have later been reported from the U.S.A. as well (Peterson et al. 1970)

The prevalence of the risk factors mentioned above is great in a normal population and various combinations of risk factors are present in numerous individuals. While many risk factors have an independent effect the combinations obviously multiply the risk.

III MATERIAL AND METHODS

1 STUDY POPULATION AND AREA

The City of Helsinki

Helsinki is the capital of Finland, and also its largest city. It is located on the southern coast of the country. The area of the city is 176.9 km². The length in the north—south direction is 17.5 km and in the east—west direction 18.6 km. Fig 1. is the map showing the area of the city. In it the hospitals with an emergency reception for patients suffering from heart attacks have been indicated.

Being the capital, Helsinki is the centre of administration, but trade, industry and education are also well represented.

The annual mean temperature in Helsinki in 1970 was 4.0 C, the highest 28.8 and the lowest —28.3. The mean relative humidity was 80 per cent.

The average population of Helsinki during the year 1970 was 522 929 (Table 1). This implies that about 12 per cent of the population of Finland resided in Helsinki in 1970. During the most recent years the total city population has increased by about one per cent yearly but the figure has remained unchanged or even decreased in some middle-aged groups: one of them — the 50—59 age group during the last five years. This is due to the fact that middle-aged people emigrate in considerable numbers, mainly to the rapidly growing, modern communities adjacent to Helsinki.

Table 1 *Mean population f Helsinki by ge and sex in th yea 1970*

Age	Males	Females	Total
—19	69337	69309	138646
20—24	26415	33311	59726
25—29	24604	27160	51764
30—34	18736	19636	38372
35—39	15892	16718	32610
40—44	15156	16810	31966
45—49	13363	17692	31055
50—54	11479	16521	28000
55—59	12071	18402	30473
60—64	10524	17833	28357
65—69	7150	13829	20979
70—74	4260	10546	14806
75—	3700	12475	16175
	232687	290242	522929

Health Services

In 1970 there were 1716 physicians in Helsinki, not all of them in practice. This is more than a third of all physicians in Finland.

In Finland it is the rule to send a patient to a hospital whenever suspicion of a myocardial infarction arises. The few exceptions are those cases where diagnosis is not made before the acute phase of the attack has passed.

Altogether 11 hospitals with departments of medicine have facilities for the care of patients with myocardial infarction. The largest is the Meilahti Hospital of the University Central Hospital, which comprises three separate medical departments. The fourth medical department of the University Central Hospital has a separate location. The city of Helsinki owns four hospitals which have a medical department. Kivelä, Maria, Malmi and Aurora Hospitals, furthermore a large hospital for the chronically ill (mainly geriatric) patients. There are also four

private hospitals, one Military Central Hospital and a ward at the Institute of Occupational Health, each of which can potentially take care of myocardial infarction patients.

An emergency reception open day and night can be found in only four of the hospitals Meilahti, Kivelä, Maria and Malmi (Fig 1) These hospitals treat more than 90 per cent of the hospitalized myocardial infarction patients in the city for obvious reasons. As can be seen from Fig. 1 three of these four hospitals are located quite close to each other in the centre of the city· only the smallest hospital, that of Malmi, lies in the north-eastern part of the city

Only two hospitals have a coronary care unit. In the Meilahti hospital, up to eight beds were reserved for coronary patients, and four in the Kivelä hospital.

There is an Alarm Centre providing doctors for house calls around the clock. After office hours five doctors as a rule are available for home visits in different parts of the city Telephone calls made to the Alarm Centre are answered by a nurse, who has authority to dispatch an ambulance for transport or who may advise immediate transportation of the patient to a hospital. Home visits are usually made in the order of the calls received, but it has been a rule to give priority to patients suspected of heart attack. There was no direct contact by radio telephone with the cars of the doctors on duty· therefore immediate contact with them at any time has not been possible. At normal times the Alarm Centre arranges for approximately 100 house calls daily

The ambulance service in Helsinki is organized in connection with the Fire Service of the city The ambulances are stationed at four different points, but with a joint telephone exchange As a rule, a doctor's or nurse's authorization is required for any ambulance transport, but in acute situations, which specification is met by patients suspected of suffering from a heart attack,

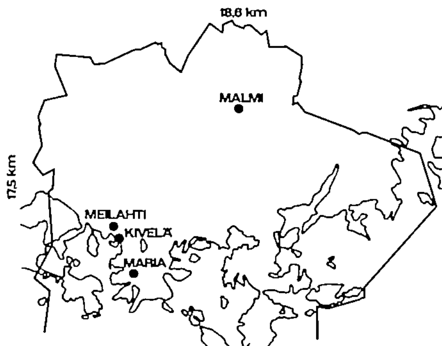


Fig. 1 Map, showing the extent of the city of Helsinki area and locations of the four hospitals having an emergency reception.

urgent transportation is carried out without authorization by any member of the medical profession. In addition to the municipal system, there are several private ambulance companies, some of them also on call around the clock.

Finland has a national health insurance scheme, and all persons under the age of 65 receive a per diem allowance if a doctor prescribes sick leave for longer than 7 week days provided that they are not already recipients of a disability pension.

2. THE ISCHAEMIC HEART DISEASE REGISTER

General outlines of the study

An Ischaemic Heart Disease Register was established in Helsinki, starting September 1st, 1969 as a part of the research programme of the Finnish Heart Association (Siltanen 1973) The design of the study and the outlines and principles followed are those proposed by the World Health Organization and consistent with other ischaemic heart disease registers in various countries, incorporated in the WHO ischaemic heart disease register programme. The study programme is documented in a series of reports prepared by working groups convened by the Regional Office for Europe of WHO (Working Group of Ischaemic Heart Disease Registers, EURO 5010 (1) 1968, (2), 1969, (4), 1970 and (5), 1971). From the very beginning there has been close co-operation with the Regional Office for Europe of the World Health Organization.

The aim of the project is to collect more and better information concerning the most severe forms of acute ischaemic heart disease, myocardial infarction and sudden death as they occur throughout the community According to the recommendation of WHO any patient should be registered whenever there is suspicion of a myocardial infarction or sudden coronary death. Every effort was made to achieve as complete registration as was possible.

The study population consisted of the registered population of Helsinki under the age of 66. Those cases were also included where the attack occurred while the patient was not within the city limits.

The age limit was considered inevitable from the beginning for various reasons. Although aged persons only comprise ten per cent of the population of Helsinki, it could be estimated that extending the registration to include all age groups would have doubled the number of cases registered and the available personnel would have been unable to cope with the task. It is also well-known that even further difficulties in diagnosis of AIHD are encountered in the aged than in the young It was also considered difficult to achieve an equally complete registration in the older age groups as in the younger ones, and it is also thought that the reliability of the information gained is less. Therefore, the registration was extended to all age groups merely over a short period.

The registration started on September 1st, 1969 as a pre-pilot study during which the propriety of the recording system was tested. After four months of this, the main study commenced at the beginning of 1970 without a break. The actual study period lasted for one year from January 1st to December 31st, 1970. The study period covering all age groups was from September 16th to December 31st, 1970. The second half of September was considered as a pre-pilot period and the incidence figures for older age groups are calculated from the three month period October—December 1970

The personnel of the Ischaemic Heart Disease Register consisted of two, sometimes three interviewing nurses and one secretary with a physician in charge. The Register had an office in connection with the Finnish Heart Association's Research Unit.

This particular study is centred on one

important area in the field of the acute ischaemic heart disease, namely that of sudden death. However all the other cases found in the Register provide the requisite background, against which comparisons of various findings are made.

Sources of information

It was considered essential to the study to make sure that the highest possible per centage of relevant cases was indeed recorded. Therefore, close co-operation was sought with all persons, institutes and au thorities from whom aid could be received to achieve this aim.

All hospitals in the city having wards for internal medicine were visited by the author and his coworkers. These hospitals are men tioned in the preceding chapter The aim of the study was pointed to and promises of co-operation were exacted. In each hospi tal one of the physicians was appointed to be liaison. Special attention was paid to ensure collaboration of the personnel in the four outpatient departments of the hospitals with a 24-hour duty which are those through which most of the hospitalized patients passed. A circular letter addressed to the physicians and to the nurses in charge was delivered to all departments of internal medicine in every hospital A further copy went to the filing staff of each hospital. The author paid visit in person to most of the wards, to all outpatient departments and to all files before or at the beginning of the pre-pilot study Good co-operation was achieved in all but one hospital in this exceptional instance too satisfactory ar rangements could be made for registration before the main study was undertaken.

Circulars, as well as articles in the local medical journals, were employed to make all physicians in Helsinki and in its environs aware of the study by which they were requested to report all patients under the age of 66 years whose case suggested possible myocardial infarction or who met with sudden death. This could be done by post or telephone an automatic recorder after office hours being provided to make the reporting as convenient as possible.

The Alarm Centre dispatching doctors on duty on house calls was advised of the study All physicians thus sent out by the Alarm Centre were requested to report the cases which they encountered. In addition, a special dispatching form was distributed to the physicians, requesting that it be used whenever a patient was sent to hospital under suspicion of a myocardial infarction. Since telephone calls received at the Alarm Centre are scrupulously clocked, reliable fixing of the time of a request for medical aid was possible.

All departments of pathology and forensic medicine in Helsinki performing autopsies were visited in person and informed of the study A special report form was distributed to the pathologists with the request that it be completed whenever a fresh myocardial infarction was found or in case ischaemic heart disease was considered to be an essential cause of death. Most of the autopsies of sudden death cases occurring outside hospitals are made at the Department of Forensic Medicine, University of Helsinki, where a member of the register team was working at that time. He made regular checks of all autopsies performed at the department.

The Police gave a daily report on all medically unattended deaths outside hospitals of which they were notified. Finnish law prescribes that all such cases have to be reported to the Police. A police file is made of them, and the police are also authorized to order an autopsy

The National Pensions Institute, which also administrates the health insurance system of Finland, made available all those cer tificates submitted to it to substantiate daily allowance claims in which a diagnosis referring to ischaemic heart disease (ICD code

410—412) appeared. Copies of relevant certificates were sent to the Register regularly

The City's ambulance service and the private ambulance companies were contacted. The clocking of the calls by the ambulance centre and the times when patients were reached were made available to the Register

Full support was also given to the Register by the city authorities for public health.

Work procedure of the IHD Register

Abundant co-operation was promised at the beginning by all parties concerned. However it was soon found that the reporting carried out by the physicians was far from complete. There were also failures on the hospitals' side. It was therefore made standard practice adopted during the first weeks of the pre-pilot study that the nurses of the Register paid regular visits to the outpatient departments of the hospitals on a 24-hour duty basis and checked all diagnoses on admission made during the time between visits. Daily visits were paid to the largest hospital (University Central Hospital Meilahti) and the other hospitals were visited 2—3 times weekly Furthermore, regular visits were made to all wards of these hospitals in order to check whether all cases had indeed been registered. Telephone inquiries constituted the means by which efforts were made to maintain co-operation with the other hospitals.

The death certificates were checked twice every month, and all relevant information was derived from them. The closest relative, if known, was then approached by letter requesting an interview and distinctly stating its purpose If no relatives were known, the official population register was checked if necessary the address was obtained from the Address Off ce After approximately one week, one of the nurses made contact by telephone or in person. If no information was available the police reports concerning those cases of which the police were notified were checked. If necessary the doctor who had signed the death certificate, or persons who had witnessed the death or any others with relevant information were contacted. A considerable number of those who died outside any hospital were recorded at the Department of Forensic Medicine before their death certificate was available.

The medical certificates substantiating claims for per diem sickness allowances of which copies were regularly received by the Register revealed some of those patients who had not been registered before. This was often the sole source of data causing inclusion in the register when the onset of illness had occurred outside Helsinki and the patient had survived. The patient was contacted at the earliest possible time after registration.

The staff of the Register carried out all the interviews, and the nurses also checked the information contained in hospital records. In each instance a special record form was completed. This procedure is common to all ischaemic heart disease registers incorporated in the programme of WHO although additions indicated by local requirements have been made. The procedure was followed through, no matter whether the patient was alive or dead after the interview any revisions and additions were made, and the time intervals involved were elicited from various sources. The interviews of hospitalized patients were mainly made at the hospital as soon as the patient's clinical status allowed. Most of the interviews of dead patients' relatives were made at their homes only relatively few were conducted at the Office of the Register Some few interviews were made by telephone if the relatives declined to have an interviewer visit them. The intervening time between the death and the interview varied greatly Most interviews were made within one month after death, but there were also longer intervals.

All registered cases were followed up for 4 weeks from onset of the acute attack, or until their death. After this period the in terview record forms and all relevant in formation were gathered together· the author personally checked the interview records, hospitals records and autopsy reports and classified the diagnosis made of the acute attack according to the principles of WHO which are set forth later

On conclusion of the study year all death certificates from this year which had accumulated in the Central Statistical Office of the State were checked once again. Six cases not registered before were then found. Furthermore all diagnoses on discharge from the University Central Hospital during the above-mentioned year were listed by computer· the same procedure was applied at the Statistical Office of the City of Helsinki concerning all city-owned hospitals. By means of this check, hospitals treating more than 90 per cent of all hospitalized myocardial infarction patients in Helsinki were covered. 48 cases not registered before were thus found.

Highly satisfactory co-operation was achieved both with patients and with the relatives of dead persons. Refusals for in terview were very few It is natural that it was more difficult to gain information concerning those who had died, only a police report was available in some instances. But the relatives of dead persons, when con tacted, invariably displayed a fully positive attitude towards the study and were highly co-operative.

3. DEFINITIONS

Acute ischaemic heart disease (AIHD)

Acute ischaemic heart disease is defined as an episode fulfilling the criteria of WHO which are stated below for one of the three diagnostic categories definite acute myocardial infarction, possible myocardial infarction, or insufficient data. An acute attack was considered to last up to 4 weeks. Any recurring attack, or death later than within four weeks, was considered a new event.

Sudden coronary death (SCD)

Sudden coronary death is defined as being an acute attack of ischaemic heart disease terminating fatally within one hour from the onset of the symptoms. The attack itself is required to fulfill the minimum criteria of AIHD given by WHO but it has to be realized that all sudden fatalities fulfill the criteria of the insufficient data category when no other diagnosis is obvious.

According to the definition, these deaths are witnessed, although there are a few exceptions. Some cases could be included in the group of sudden deaths notwithstand ing the fact that the death was unwitnessed. Examples of this are those cases in which the victim had been seen in a normal condi tion and had been found dead soon after wards. Cases in which a patient hospitalized for other causes died suddenly in hospital have been included in the SCD group

Non sudden coronary death (NSCD)

Non-sudden coronary death is defined as an acute attack terminating fatally within the first 28 days of the onset of symptoms, but not within the first hour The attack is required to fulfill the minimum criteria of AIHD given by WHO Unwitnessed deaths with unknown duration of the fatal attack were not included in this group either

Unwitnessed death

Unwitnessed death is defined as a fatal attack of AIHD which fulfills the criteria of AIHD given by WHO but in which the terminal episode is not witnessed and the duration of the attack is therefore not known.

Survivors (SURV)

As survivors those patients with AIHD are considered who survived the first 28 days after onset of the acute attack.

Diagnostic categories

Four weeks after the onset of symptoms all registered cases were assigned to the dif ferent diagnostic categories on the basis of available information, and according to the criteria given by WHO at that time (Working Group of Ischaemic Heart Disease Registers, EURO 5010 (4), 1970). Minor revisions by WHO have later transpired concerning assignment to the categories Possible myocardial infarction or Insufficient data whereas the minimum criteria of acute ischaemic heart disease and the criteria for definite myocardial infarction have remained unchanged.

The registered episode was diagnosed on the basis of four criteria history of the acute attack, electrocardiograms, serum enzyme levels, and post mortem examination. Quite naturally information concerning all four factors was not always available. In many instances of sudden death there was nothing but the result of post mortem examination, if any Those cases are also numerous in which a history of acute chest pain was recorded while electrocardiogram and serum enzyme levels were usually absent. The four diagnostic categories and their definitions are listed below

1 Definite acute myocardial infarction
   — Unequivocal ECG evidence of recent myocardial infarction, o
   — Equivocal ECG evidence of recent myocardial infarction with elevated levels of appropriate serum enzymes, or
   — Elevated levels of appropriate serum enzymes with a typical history of chest pain, or
   — Positive post-mortem evidence
2. Possible acute myocardial infarction.
   — A typical history of pain without evidence justifying assignment of the case to the first category
3. Insufficient data.
   — Insufficient items of evidence justifying assignment to the above two categories, while no diagnosis other than that of ischaemic heart disease has been made. Most cases of sudden death belong to this category
4. No myocardial infarction.
   — Cases in which another diagnosis was made or which did not fulfill the criteria for assignment to any one of the above-mentioned three categories. These cases were excluded from the study

The definitions for unequivocal and equiv ocal ECG changes have been given by WHO earlier (Hypertension and coronary heart disease, 1959 and Working Group of Ischaemic Heart Disease Registers. EURO 5010 (4), 1970).

The following definition for elevated serum enzyme levels was applied in this study SGOT 30 IU/ml or more, or SGOT 20—29 if LDH was over 240 and isoenzymes 1 and 2 were elevated. The LDH isoenzymes were, however determined in exceptional cases only

The definition for the typical history of chest pain is defined by WHO earlier (Arterial hypertension and ischaemic heart disease, 1962).

The definition for positive post-mortem evidence of an acute myocardial infarction in this study according to WHO is. The presence of a fresh myocardial infarction and or the presence of a recent thrombotic occlusion of a coronary artery observable by the naked eye. Only the naked eye appearance counted as positive evidence.

Occlusion, stenosis or calcification of the coronary arteries, scars after old infarctions, fibrosis etc. were also recorded It is clear

that they furnished valuable aid in the determination of the cause of death, even though actual acute changes were absent.

Age

The age at onset of symptoms.

Marital status

The subjects are divided into married, single divorced and widowed persons.

Social class

The division into *four* social classes is based on the subjects occupation. The same division is used in the census in Finland.

Class I. Comprises professionals, higher officials, managers etc.

Class II Consists of employers in small enterprises, the self-employed, technicians, foremen, higher clerical workers, nurses etc.

Class III Consists of skilled workers, lower grade clerical employees, shop assistants, drivers, and workers on a similar level etc.

Class IV Consists of various kinds of unskilled workers, domestic helps, char women, porters etc.

The retired persons were classified according to their previous occupation. The final analysis of the association of acute ischaemic heart disease and social class was made both including and excluding the retired persons.

Housewives are classified according to their own occupation if possible. Those with no occupation of their own are referred to the social class of their husbands and widows to that of their late husband.

Premonitory symptoms

The patients and their relatives were questioned as to premonitory symptoms during 28 days preceding the onset of the attack. The following symptoms were particularly inquired after: the fresh occurrence of angina pectoris or the exacerbation of existing angina pectoris discomfort in the chest, heaviness of the arms, nausea, unusual tiredness, unusual dyspnoea and the first occurrence of palpitations. Other specific symptoms reported to the interviewer were also recorded. Symptoms with onset earlier than 4 weeks before the attack were not recorded unless distinct exacerbation of the symptom had been noted during the preceding month. Visits to a doctor within 1 2 or 4 weeks before onset of the attack were recorded together with the cause of each visit. Of several visits made during the preceding month only the last one was recorded.

Previous diseases

The information on previous diseases was gained by the interviewers from the patients or their relatives, and from hospital records. Whenever possible the hospital case records of earlier hospitalizations were also checked. According to the recommendation by WHO any history of myocardial infarction, hyper tension, stroke or diabetes mellitus was divided into two validity categories con firmed if there were hospital case records or other medical documents confirming the patient s history and non-confirmed if no such confirmation could be found but if the patient s history was consistent with the diag nosis. In the presentation of results these two validity categories were combined.

Myocardial infarction

A confirmed myocardial infarction included in the patient s history was required to fulfill the diagnostic criteria laid down in this study for a definite myocardial infarction. If con firmation was not possible even though the history was consistent with the diagnosis, a non-confirmed episode was recorded. In those cases where more than one myocardial infarction had been reported, only the con firmed episodes were taken into account.

Angina Pectoris

The previous definition of WHO (Arterial hypertension and ischaemic heart disease 1962) for effort angina constituted the basis. A history of chest pain was accepted as positive if a physician's opinion stated that angina pectoris was concerned. If it appeared to the author that the documented description of chest pain contained in the interview record form was compatible with typical effort angina, this was also accepted as a positive symptom.

Hypertension

If diagnosis of hypertension had previously been made by a physician, the history was accepted as positive, either confirmed or non-confirmed, depending on the availability of confirming documents. Hypertension detected after admission owing to the registered episode was not included.

Other cardiovascular diseases

This category consists of diseases such as a stroke, valvular heart disease, pulmonary heart disease and chronic disturbance of the cardiac rhythm. Congestive heart failure was also included. The group constitutes, therefore, a heterogeneous assembly of diseases, part of which no doubt represent symptoms of ischaemic heart disease or of other cardiovascular diseases.

Diabetes mellitus

The diagnosis of diabetes mellitus was accepted as confirmed if it was confirmed by previous medical documents. Constant antidiabetic treatment was also accepted as positive proof. If the patient's history was unsupported, it was considered as being non confirmed.

Obesity

The height and weight of the patients were obtained from medical records in the hospitals or by inquiry from the patients or their relatives. In some cases only measurements made in connection with autopsy were available.

As an index of relative weight a body mass index, that is Quitelet's index, weight/height-squared was used.

Smoking habit

The patients were first divided into three categories current smokers, ex-smokers and non-smokers.

The definitions for the different categories are

A smoker is one who smokes at least one cigarette per day (or an equivalent quantity of pipe or cigar tobacco) or who has smoked at this level up to a time later than three months before the acute attack.

An ex-smoker is one who has smoked at the level defined above but has given up smoking not later than three months before the acute episode.

A non-smoker is one who has never smoked tobacco at the levels indicated above.

The cigarette smokers were further divided according to the number of cigarettes consumed daily while the pipe and cigar smokers constituted a category of their own. Those smoking both cigarettes and pipe or cigars were included in the group of cigarette smokers.

Physical activity

A particular questionnaire was completed for every patient concerning their daily activity on the job, the time spent walking, sitting or standing, their means of getting to and from work, the frequency of lifting or carrying heavy objects, etc. A similar set of questions was asked concerning the pa

tients' off the-job activities, the indulging in sport, walking gardening and household work.

On the basis of the questionnaires, the author assigned the patients to three activity categories with regard to the on-the-job and off the-job activities — both separately

On the job activity

Class I Inactive group. Mainly sitting Never or very rarely lifting or carrying heavy objects.

Class II Medium group Work also comprising walking. Occasional lifting or carrying of heavy objects.

Class III Active group More walking than sitting Frequent carrying or lifting of heavy objects. Tasks causing breathlessness or sweating are frequent.

Off the job activity

Class I Inactive group. Virtually no physical activity during leisure time.

Class II Medium group Walking cycling or moderate physical work at home at least 4 hours weekly

Class III Active group Sports or heavy work at home regularly at least 4 hours weekly

An attempt was made to test the validity of the questionnaire by interviewing a group of 87 coronary heart disease patients of the same age who did not belong to the study material. In addition to the questionnaire employed in this study a recall record form was used and the mean calory expenditure was calculated for each one of these patients. The author assigned them to the three activity groups on the basis of the same principles which were applied to the patients of the actual study

Table 2 presents the results of this test. It is seen that most of the cases are concentrated in the inactive categories. The differences between various categories are statistically only almost significant. On the other hand, it is acknowledged that the validity of the method of comparison is questionable.

Place of onset of symptoms and of death

The place of onset of symptoms was classified as follows at work, at home, in a hospital, or in another place. In some cases there was no definite onset of symptoms and it was also naturally impossible to define the place of onset.

The place of death was classified in the same manner except that those who died during transportation to a hospital or at a hospital's outpatient department within one hour of arrival were referred to extra groups of their own.

Table 2. *Mean daily calory expenditure of 87 patients with IHD in different job-connected and leisure time-connected physical activity categories (numbers of patients in parenthesis).*

Physical activity connected with leisure time	Job-connected physical activity I		II		III		All cases	
I	2485	(10)	2543	(7)	402	(1)	2503	(18)
II	2522	(40)	2588	(4)	2398	(3)	2529	(47)
III	2639	(16)	2824	(6)	2333	(1)	2713	(22)
All cases	2544	(66)	2642	(18)	2908	(5)		

$$F = \frac{s_T^2}{s} = 1.25$$

Activity at onset of symptoms

The subjects were considered to be at rest when sleeping, lying or sitting without engaging in any activity. The category »at work» comprises a wide variability of activities involving light or moderate physical stress. The exceptional cases of strenuous activity were separately coded.

4. MATHEMATICAL TREATMENT

Of the various quantitative variables measured in the study, the means were calculated in the different groups (SCD, NSCD, SURV) separately for both sexes and, as indicated, in different age groups, and their standard deviations were determined. Statistically significant differences between such means were established by Student's t-test. A one-way analysis of variance was applied in testing the mean calory expenditure in different classes of physical activity and with regard to body mass index. The comparison of proportions in different classes was carried out by means of the usual test based on an approximation of the normal distribution.

Distributions of various aspects in the different groups (SCD, NSCD, SURV) were displayed in contingency tables of a suitable size. Statistically significant differences between the groups (SCD, NSCD, SURV) were established by the goodness of fit test, based on an approximation of the chi-square distribution (e.g. Vasama and Vartia 1972).

The original data concerning marital status, social class and physical activity were age-adjusted by a direct method of standardization (Armitage 1971). After standardization, the above-mentioned chi-square goodness of fit test was used in testing the statistical significance of the observed frequency distributions.

The statistical significance of differences is indicated by the following symbols:

N.S.D.	No statistically significant difference ($p \geq 0.05$)
*	Almost significant difference ($p < 0.05$)
**	Significant difference ($p < 0.01$)
***	Highly significant difference ($p < 0.001$)

IV RESULTS

1 REGISTERED CASES OF ACUTE ISCHAEMIC HEART DISEASE — AGE, SEX AND DIAGNOSTIC CATEGORIES

All cases of acute ischaemic heart disease in those aged 65 years or younger registered within the population of Helsinki, constitute the patients in the present study Some of their basic characteristics are described in the following

Results

Altogether 1267 cases fulfilling the minimum criteria of WHO for AIHD were registered. 953 (75.2 per cent) were males and 314 (24.8 per cent) were females. Table 3 and Fig 2 present the patients by age, sex and diag nostic category of WHO as defined on page 25 The number of cases in both sexes increases rapidly with age. The mean age of females was higher (57.9 years) than that of males (54.9 years) The different age distribution between both sexes is clearly evident from Fig. 2, the majority of female cases being concentrated in the oldest age groups.

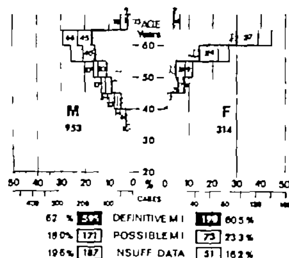


Fig. 2. Age and sex distributions of acute ischaemic heart disease cases in different diag nostic categories of WHO numbers of cases and percentages of male and femal cases in each category

Table 3. *Distribution of acut ischaemic heart disease cases by age sex and WHO diagnostic category*

Age	Definite	Possible	Insuff data	Total
		Males		
—29	1	—	—	1
30—34	7	—	—	7
35—39	12	10	6	28
40—44	48	14	12	74
45—49	85	24	17	126
50—54	104	33	17	154
55—59	152	40	51	243
60—64	163	45	66	274
65	23	5	18	46
Total	595	171	187	953
Per cent	62.4	18 0	19.6	100 0
		Fem les		
—29	2	—	—	2
30—34	1	—	—	1
35—39	—	1	1	2
40—44	1	1	2	4
45—49	18	7	1	26
50—54	16	11	9	36
55—59	46	24	14	84
60—64	95	27	18	140
65	11	2	6	19
Total	190	73	51	314
Per cent	60 5	23.3	16.2	100 0

Table 3 and Fig 2 also reveal the relative proportions of the different WHO categories in both sexes. 62.4 per cent of male and 60.5 per cent of female cases fulfilled the criteria of definite myocardial infarction. The relative proportion of all WHO categories is roughly the same in different age groups.

Table 4 shows the age and sex composition of the study group of 239 sudden coronary death cases (SCD) in juxtaposition with the two comparison groups, that of 240 non sudden coronary death cases (NSCD) and that of 736 survivors (SURV). The small group of 52 unwitnessed deaths is also presented. The proportion of dead cases from all AIHD cases in different age groups is presented in Fig. 3 which indicates also the case fatality rate, 43 6 per cent for males and 37 1 per cent for females. In Fig 4, the age and sex composition of all fatal cases of AIHD is presented in three subgroups sudden deaths, non-sudden deaths and unwitnessed deaths.

It is noted that SCD cases show an even greater male prominence (83 7 per cent) than all AIHD cases (75.1 per cent) this is especially noteworthy in the youngest age groups. There are no female sudden death cases under the age of 40 years, in contrast to six male cases, and only one female case occurs in the next age group, from 40 to 44 years, as opposed to ten males.

In each one of the comparison groups the mean age for males is lower than that for females. The males mean ag is closely equal in all groups consisting of deceased persons, 56.1 years in the NSCD group and in the group of unwitnessed deaths and 56.2 years in the SCD group It is definitely lower in the SURV group (53.9). The females' mean age is highest in the group of un witnessed deaths, 59.5 years, followed by the NSCD group, 59.2 years. The mean age is lower in the SURV group (57.5) and lowest in the SCD group (57.2 years). If both sexes are combined, it can be found that the mean age is definitely lowest in the SURV group (54.9 years), followed by the SCD group (56.3 years) The mean age in the group of unwitnessed deaths is even *higher (57 0 years).*

Sudden death constituted 18.8 per cent of all registered cases of AIHD 21.0 per cent of male and 12.4 per cent of female cases. The male female ratio of all cases of AIHD was 3 1 that of all fatal cases of AIHD was 4 1 but in the SCD group it had the ratio 5 1.

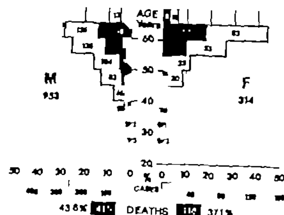


Fig. 3 Age and sex distributions of acute ischaemic heart disease cases, among survivors *and fatal cases* numbers of cases and percentages of male and femal fatal cases.

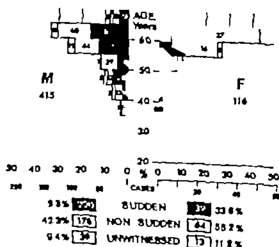


Fig. 4 Age and sex distributions of fatal acute ischaemic heart disease cases in SCD, NSCD and unwitnessed death groups numbers f cases and percentages of male and female cases in each group.

Comments

Considering the number of registered cases of AIHD it must be understood that only persons in the working age group are concerned. The estimated number of AIHD in the age groups older than 65 calculated from the three-month study period, consisted of 1312 cases. This means that the persons of working age forming the object of this study only constitute half of the total burden of AIHD borne by the health services and by the medical care system of the community.

The percentage of the registered cases per month increased approximately by ten from the pre-pilot study period, probably owing to more complete coverage after the registration system had become firmly established. The checks on the perfection of the registration disclosed a total of 54 cases which had escaped notice. This is 4.5 per cent of all registered cases. It is likely that those who die in AIHD are more positively registered than any others considering that the law prescribes that any death certificates have to be sent to the city authorities independent of the resident's place of death, and that all death certificates are gathered at one point. The registration of survivors has probably not been equally complete. The checking process probably covered the majority though not all, of the cases hospitalized in Helsinki. The cases treated outside Helsinki were registered if prescribed sick leave and if they were not already receiving a disability pension on the basis of substantiating claims for a per diem allowance. Only a few surviving patients with AIHD entered the Register from other sources, reported, for instance, by their attending doctor. Therefore it must be considered that a certain number of cases has escaped notice. In Finland, nearly all patients in whom myocardial infarction is even suspected are sent to a hospital. Almost the only exception consists of cases which have eluded diagnosis before the acute phase has

Table 4. *Age and sex distribution of acute ischaemic heart disease cases in different groups.*

Age	Sudden deaths			Non-sudden deaths			Unwitnessed deaths			Survivors			All AIHD cases		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
—29	—	—	—	—	—	—	—	—	—	1	2	3	1	2	3
30—34	2	—	2	—	—	—	—	—	—	5	1	6	7	1	8
35—39	4	—	4	8	1	9	1	—	1	15	1	16	28	2	30
40—44	10	1	11	13	—	13	5	1	6	46	2	48	74	4	78
45—49	28	3	31	10	3	13	5	—	5	83	20	103	126	26	152
50—54	20	6	26	29	3	32	1	2	3	104	25	120	154	36	190
55—59	53	13	66	44	16	60	11	2	13	135	53	188	243	84	327
60—64	67	13	80	60	37	97	11	7	18	136	83	219	274	140	414
65	16	3	19	12	4	16	5	1	6	13	11	24	46	19	65
Total	200	39	219	176	64	241	39	13	52	538	198	736	953	314	1267
Per cent	83.7	16.3		73.0	27.0		75.0	25.0		73.1	26.9		75.1	24.9	

Table 8. *Distribution of acute ischaemic heart disease cases in different groups by age, sex and WHO diagnostic category.*

Age	Sudden deaths				Non-sudden deaths				Unwitnessed deaths				Survivors				All AIHD cases			
	Def.	Poss.	Insuff. data	Tot.	Def.	Poss.	Insuff. data	Tot.	Def.	Poss.	Insuff. data	Tot.	Def.	Poss.	Insuff. data	Tot.	Def.	Poss.	Insuff. data	Tot.
Males																				
—29	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	1	—	—	1
30—34	2	—	—	2	—	—	—	—	—	—	—	—	5	—	—	5	7	—	—	7
35—39	1	—	3	4	4	2	2	8	—	—	1	1	7	8	—	15	12	10	6	28
40—44	5	3	2	10	5	1	7	13	2	—	3	5	36	10	—	46	48	14	12	74
45—49	12	5	11	28	7	2	1	10	1	—	4	5	65	17	1	83	85	24	17	126
50—54	6	3	11	20	21	3	5	29	—	—	1	1	77	27	—	104	104	33	17	154
55—59	12	6	35	53	35	4	5	44	1	—	10	11	104	30	1	135	152	40	51	243
60—64	12	9	46	67	39	10	11	60	2	—	9	11	110	26	—	136	163	45	66	274
65	4	2	10	16	7	—	5	12	2	—	3	5	10	3	—	13	23	5	18	46
Total	54	28	118	200	118	22	36	176	8	—	31	39	415	121	2	538	595	171	187	953
Per cent	27	14	59		67	13	20		21	0	79		77	22	1		62	18	20	
Females																				
—29	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2	2	—	—	2
30—34	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	—	—	1
35—39	—	—	—	—	—	—	1	1	—	—	—	—	—	1	—	1	—	1	1	2
40—44	—	—	1	1	—	—	—	—	—	—	1	1	1	1	—	2	1	1	2	4
45—49	2	1	—	3	3	—	—	3	—	—	—	—	13	6	1	20	18	7	1	26
50—54	—	—	6	6	2	—	1	3	—	—	2	2	14	11	—	25	16	11	9	36
55—59	4	1	8	13	8	3	5	16	1	—	1	2	33	20	—	53	46	24	14	84
60—64	3	2	8	13	28	4	5	37	2	—	5	7	62	21	—	83	95	27	18	140
65	—	—	3	3	2	—	2	4	—	—	1	1	9	2	—	11	11	2	6	19
Total	9	4	26	39	43	7	14	64	3	—	10	13	134	63	1	198	190	73	51	314
Per cent	23	10	67		68	11	21		23	0	78		68	32	1		60	23	16	

passed. Of these cases, too reports should be received by way of the certificates for substantiating per diem claims, with the same reservation as has been mentioned above.

To summarize the foregoing, it can be said that obviously the registration of fatal cases has been fairly complete, while that of survivors has been less perfect. Therefore the mortality figures may be slightly exaggerated. The checking procedure which was undertaken justifies the assumption that this over-estimation cannot be of any substantial order It is natural that cases with unrecognized myocardial infarctions are never discovered in a study of this kind. According to several prospective epidemiological studies, the number of such cases seems fairly substantial (Rosenman et al. 1967 a, Master and Geller 1969 Kannel et al. 1970)

The definitions of the different groups, i.e., sudden coronary deaths (SCD), non-sudden coronary deaths (NSCD) and survivors (SURV), and that of unwitnessed deaths have been given earlier (pages 24—25). The group of unwitnessed deaths posed a special problem. It is likely that they represent, in part, sudden deaths and non-sudden deaths, in part, but the relative proportions of both are not known. Therefore, it was not considered proper to include them in either one of the above-mentioned groups. Their number is comparatively small, only 52 cases, i.e., 4.1 per cent of all AIHD cases. In the subsequent analyses, this group is included in the total of AIHD cases and it is treated as a separate group, while the results referring to it are not stated in all instances.

The results show that AIHD is more common among males than among females. The attack afflicts males at younger ages than it does females. The definitely lower mean age of the survivors, compared with those who die during the attack, can be interpreted to mean that age is a factor increasing the mortality rate

Table 5 shows that according to the criteria of WHO the validity of the diagnosis of AIHD can be considered best in the SURV group, followed by the NSCD group it is better in both groups than in the SCD group This is hardly unexpected and depends on different possibilities as regards confirming the diagnosis in the different groups. The basis of the diagnosis in the SCD group will be discussed in detail in Chapter IV.3

Summary

1267 cases of AIHD in persons aged 65 years or younger were registered during the study year However this is only half of all cases provided all ages are considered. 239 cases belonged to the SCD group 240 to the NSCD group 52 cases to the group of unwitnessed deaths and 736 cases to the SURV group The case fatality rate was 43 6 per cent for males and 37 1. per cent for females. Male prominence was obvious in all comparison groups. It was most accentuated in the SCD group The mean age of females was 57.9 years and that of males 54.9 years. 62 4 per cent of male and 60.5 per cent of female cases fulfilled the criteria of definite myocardial infarction. Sudden death constituted 18.8 per cent of all registered cases of AIHD 21 0 per cent of male and 12.4 per cent of female cases.

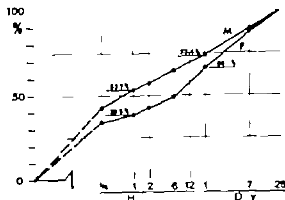
2. RELATIVE PROPORTION OF SUDDEN DEATHS FROM ALL ACUTE ISCHAEMIC HEART DISEASE DEATHS AND INCIDENCE OF SUDDEN CORONARY DEATH

In considering the frequency of sudden coronary death in a community two different approaches are possible On one hand the mortality of AIHD may be considered as a function of time and the relative number of sudden deaths, with reference to all AIHD deaths or to all AIHD cases,

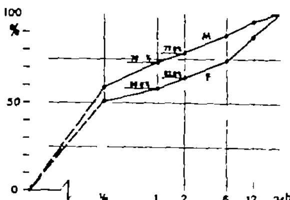
can be determined. On the other hand, one may calculate the incidence of sudden coronary death. Both procedures enable the results to be compared with results from other comparable studies. In the following an attempt has been made to apply both manners of evaluating the frequency of SCD in the present study

Results

In altogether 479 fatal cases of AIHD in 376 male and 103 female cases, the time interval from the onset of symptoms to death was known. Fig 5 showing the cumulative mortality of both sexes as a function of time reveals a high acute mortality in AIHD attacks. At the cut point of this study at one hour after the onset of symptoms, the different course of AIHD attack of males and of females is clearly evident. 53.1 per cent of the males who died during the first four weeks after the attack, as opposed to only 38.5 per cent of such females, died within the first hour. If only those cases are considered in which death occurs within 24 hours, the critical importance of the first hour is also very clearly discernible (Fig 6).



F g 5 Cumulative mortality graphs f mal and female patients with acute ischaemic heart disease who died within 28 days, according to interval from onset of ttack to death.



F g. 6 Cumulative mortality graphs of male and female patients with acute ischaemic heart disease who died within 24 hours, according to interval from onset of attack to death.

72.4 per cent of these males and 58.0 per cent of these females were dead after the first hour had passed. Figures 5 and 6 also reveal that most of the deaths occurring within one hour are in actual fact momentaneous, or at least terminate within 15 minutes.

If those cases are also taken into account where the exact time interval from the onset of symptoms is not known, there were altogether 531 deaths in AIHD within four weeks: 415 (78.2 per cent) of them males and 116 (21.8 per cent) females. Sudden death constituted 44.9 per cent of these deaths (48.2 per cent of the males and 32.6 per cent of the females).

Fig. 7 shows the incidence of sudden coronary death as well as that of AIHD for both sexes as a function of age. It can be seen that all four incidence graphs (presented in logarithmic ordinates) constantly ascend with age. Both female incidence curves run throughout on a lower level than the corresponding male curves. The sex difference is more prominent in the case of SCD. While the female incidence of AIHD reaches the males' level with a delay of roughly ten years, the corresponding time interval is closer to 15 years for the sudden death graphs.

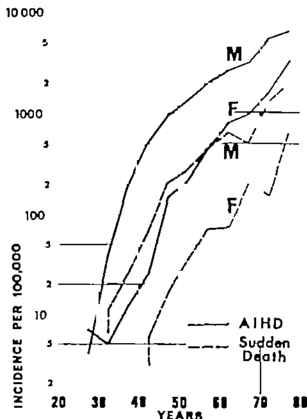


Fig. 7 Age-dependent incidence of acute ischaemic heart disease and of sudden coronary death in males and females, per 100 000 inhabitants of Helsinki. Parts of the graphs (age groups over 65 years) based on three month study period distinguished by thinner lines.

Comments

Several community and prospective studies have consistently pointed out the critical significance of the first hours in an attack of acute ischaemic heart disease. Within one hour 38 to 75 per cent of all AIHD deaths occur

In the Edinburgh Community Study death within one hour constituted 47.6 per cent of fatal male and 38.9 per cent of fatal female cases (Fulton et al. 1969). In Belfast, the corresponding figures were 40 7 per cent for males and 40 4 per cent for females, calculated from all IHD deaths within 4 weeks with known duration of the last attack (McNeilly and Pemberton 1968) In Framingham, 57.6 per cent of male and 38.9 per cent of female fatal cases terminated within one hour (Gordon and Kannel 1971), and in Tecumseh 45.9 per cent of fatal cases of both sexes respectively (Chiang et al. 1970). In the East West Study a prospective cardiovascular study carried out in two parts of Finland, 51 1 per cent of IHD deaths occurred within one hour (Punsar 1971) Several other studies have reported similar results (Eisenberg et al. 1961 Spiekerman et al. 1962, Bainton and Peterson 1963, Mathewson et al. 1965 Kuller et al. 1966 a, Fry 1968, McWhinney 1968 Stamler et al. 1969).

Compared to the studies cited, the results in the present study seem to be fairly similar A distinct male preponderance is seen in this study in agreement with most of the previous studies mentioned above.

Another aspect with reference to sudden coronary death is illustrated by the incidence figures. Such figures previously reported are few and they are based on studies in which widely different definitions of sudden coronary death have been applied. Results from certain studies are compiled in Table 6 Only the Framingham and Tecumseh studies employed a time interval of one hour From the Edinburgh Community Study the relative proportion of deaths occurring within one hour was reported earlier (Fulton et al. 1969), but incidence figures are only available concerning medically unattended deaths (Armstrong et al. 1972). The data from Stockholm likewise concern medically unattended deaths, deaths in hospitals or in other institutions not being included (Wikland 1971) In Baltimore, a time interval of two hours was used (Kuller et al. 1967), while in the HIP study New York, no definite time interval was stated (Weinblatt et al. 1968).

Table 6 reveals that the male incidence discovered in the present study is very close to that in Framingham and in Edinburgh. It is obviously higher than in Baltimore, New York and Stockholm. Comparison with the incidence in the Tecumseh Study (Chiang

Table 6. *Age-dependent incidence of sudden coronary death in different studies, per 1000 inhabitants per year*

M le

Age	Helsinki	Framingham	Baltimore	H.I.P New York	Stockholm	Edinburgh	Tecumseh
							M and F
30—34	0.1						
35—39	0.3	0.5			0.1		0.3
40—44	0.7				0.3		
45—49	2.1	1.5			0.6	1.2	1.0
50—54	1.7		2.0	1.4	0.7		
55—59	4.5	2.6			2.0	3.2	2.0
60—64	6.4			1.8	3.5		
65—69	5.0				6.0	5.7	5.5
70—74	13.1				7.7		
75—79	19.6				12.8		8.7

F m l

Age	Helsinki	Framingham	Baltimore	H.I.P New York	Stockholm	Edinburgh	Tecumseh
							See bove
40—44	0.1						
45—49	0.2	0.2	2.0		0.1		
50—54	0.4			0.2	0.2		
55—59	0.7	0.6			0.3	1.4	
60—64	0.7			0.6	0.9		
65—69	2.0				1.6	2.8	
70—74	1.5				2.8		
75—79	7.1				4.1		

et al. 1970) is difficult since the figures stated are concerned with both sexes however the incidence is probably higher in Tecumseh. The incidences given for females are very close only those of Edinburgh are distinctly higher Sudden coronary death is relatively rare among females and reaches a level comparable with the males in age groups which are 10 to 15 years older To summarize the foregoing, it can be stated that the present study furnished results that are on the same lines as previous studies, reported from countries such as the U.S.A. and England, where incidence of IHD in general is also high.

When the frequency of SCD is considered it should be realized, however that even though the time limit is accurately defined, the reported duration is merely a rough estimate made by persons witnessing the fatal attack. Under these circumstances, it is certainly inadmissible to assume that the relatives would be capable of a reliable determination of the duration of the fatal attack. This biasing effect is offset, to some extent, by the fact that the majority of deaths occurring within one hour are in fact momentaneous, thus definitely ranging with in the time limit given. On the other hand, in all likelihood some of the persons apparently succumbing to sudden death have actually had definite symptoms more than one hour prior to death, which they have not disclosed to their relatives.

Therefore, it appears that in spite of an accurate definition of SCD the recorded number of SCD cases is only an estimate even at its best. Owing to the nature of the problem in itself exact information is impossible to obtain. These circumstances probably encumber studies in other areas as well. The sole means by which reasonably comparable results may be achieved is to apply strictly uniform criteria and to make the study design also comparable in other respects. This will be achieved in the near future, as soon as results are available from all Ischaemic Heart Disease Registers comprised in the WHO comparative study

Summary

The suddenness of death seems to be a typical feature in fatal AIHD cases. No less than 53.1 per cent of male, as opposed to only 38.5 per cent of female fatal attacks with known duration terminated within one hour This remarkable sex difference is also reported earlier The relative proportion of SCD from all AIHD deaths as well as the incidence of SCD both seem to be high, although figures on the same level have been reported from certain other countries. The possibilities of reliable comparisons are restricted by substantial differences between the definitions employed.

3 BASIS FOR DIAGNOSIS IN CASES OF SUDDEN CORONARY DEATH

According to the rules of WHO given above the diagnosis of AIHD is based on four criteria a history of chest pain, a post mortem examination, electrocardiographic changes, and serum enzyme tests. It is natural that usually only the first two items, if any are available when the problem is to determine the cause of sudden death.

Autopsy is, no doubt the best support of the diagnosis, regardless of the fact that myocardial necrosis in experimental studies only becomes visible after some 6 to 12 hours, and that the pathological criteria for diagnosis are anything but firmly established.

A history of acute chest pain is obtained in a certain proportion of SCD cases, but remarkably often the attack is momentaneous and according to reports without symptoms.

In addition to the above-mentioned criteria, a history of previous, diagnosed ischaemic heart disease, either myocardial infarction or angina pectoris, no doubt provides strong evidence for AIHD as being the cause of sudden death. The same concerns to a certain degree prodromal symptoms during the month preceding the fatal attack, especially a recent onset or the progressive character of previous angina pectoris.

The basis employed in the present study in determining the cause of death is discussed in the following, separately for autopsied and for non-autopsied cases. Case histories are presented in those cases in which the diagnosis is considered to have remained uncertain.

Autopsied cases

In all, 192 of the total of 239 SCD cases (80.3 per cent) were autopsied, namely 159 out of 200 males (79.5 per cent) and 33 out of 39 females (84.6 per cent).

Conventional autopsy technique was applied at most autopsy places. In addition to macroscopic examination of the myocardium the coronary arteries were longitudinally dissected, a search was made for evidence of a fresh thrombus and the degree of stenosis was estimated. Microscopic in addition to macroscopic examination was very frequent. It was performed in 119 cases in all (62 per cent of the autopsied SCD cases). At the department of forensic medicine at the university where the majority of SCD cases was autopsied (78.6 per cent of all autopsied SCD cases) a more detailed method for examination of the heart pro-

posed by WHO (Working group on ischaemic heart disease registers, EURO 5010, 1969) was used during the study period. The method included e.g histochemical staining by employing a dehydrogenase reaction. The results of the staining were not, however used as a basis for diagnosis but only for the selection of histological specimens.

The autopsied cases were divided into three main categories according to the autopsy finding

1. Cases with recent myocardial infarction and/or a fresh thrombus in the coronary arteries established either in macroscopic or in microscopic examination. It is noted that only naked eye appearance is accepted as positive post mortem evidence when the cases are allocated to WHO diagnostic categories.

2. Cases with acute ischaemic changes in the heart muscle, not classifiable as acute myocardial infarction.

3 Cases showing no acute changes in the myocardium.

Scars after old myocardial infarctions and the degree of coronary arteriosclerosis were also recorded and they were considered to provide valuable support for the AIHD diag nosis in the two latter categories.

Coronary arteriosclerosis was defined as being severe provided it had progressed to total occlusion in at least one of the main coronary arteries. The grade was recorded as moderate if at least fifty per cent of the lumen was occluded and as slight if less occlusion had been discovered. Coronary arteriosclerosis was by no means absent, in these cases either

Table 7 presents the autopsy findings in the 192 autopsied SCD cases. The cases have been classified in the three main categories mentioned above and presented in a decreasing sequence according to the degree in which the autopsy finding supported the diagnosis of AIHD

It can be seen that in about 40 per cent of male and 30 per cent of female SCD

Tabl 7 *A topsied cases of sudden coronary death. Post mortem findings.*

	Recent myocardial infarct or fresh thrombosis		Acute ischaemic changes		No acute myocardial changes			
	Macroscopic diagnosis	Microscopic diagnosis	With old infarct	Without old infarct	With old infarct	Severe coronary arterio-sclerosis	Moderate coronary arterio-sclerosis	Slight coronary arterio-sclerosis
				M les				
—49	20	2	3	4	6	2	1	2
50—59	16	5	12	2	20	2	2	1
60—65	16	5	13	6	13	—	6	—
Total	52	12	28	12	39	4	9	3
Per cent	32.7	7.5	17.6	7.5	24.5	2.5	5.6	1.9
	40.2		25.1		34.7			
				F m l				
—49	2	—	—	1	—	—	—	1
50—59	4	1	1	5	2	—	3	1
60—65	3	—	3	—	4	—	1	1
Total	9	1	4	6	6	—	4	3
Per cent	27.3	3.0	12.1	18.2	18.2	—	12.1	9.1
	30.3		30.3		39.4			

cases, autopsy indicated an evidence of a fresh myocardial infarct, or a fresh thrombus in the coronary arteries. In 12 male and one female case this was only revealed by microscopic examination and these cases could not be allocated to the WHO category »definite myocardial infarction Nevertheless, the cause of death must be considered as confirmed in them, too.

Another 25 per cent of male and 30 per cent of female cases were classified in the second category showing acute ischaemic changes, such as soft consistency oedema, pallor etc. In about two-thirds of these cases myocardial scars furnished additional support for the AIHD diagnosis. However 12 male and 6 female cases presented only changes of acute ischaemia without any scars of an old infarct. Coronary arteriosclerosis was estimated to be severe in 5, moderate in 8 and slight in 5 cases. If severe coronary arteriosclerosis is considered as justifying the diagnosis of AIHD there remain 13 cases in which support for the diagnosis had to be sought from the case histories.

In 3 cases, typical chest pain was reported in connection with the fatal attack. The diagnosis of IHD was made during life in 5 cases and that of hypertension, in another 3 cases. In two cases a fresh occurrence of angina pectoris during the month preceding death was reported.

There remained only 4 cases, two males and two females, in which the coronary arteriosclerosis was of moderate or slight degree and the history did not lend any additional support for the diagnosis of AIHD. In them, an autopsy finding of acute ischaemia remained the only basis for diagnosis.

More than one-third of all autopsied SCD cases fell into the third category in which no acute changes in the myocardium could be found. In a notable percentage of these however strong evidence in favour of IHD was provided either by means of myocardial scars or by means of advanced coronary arteriosclerosis.

If scars after an old myocardial infarct and severe coronary arteriosclerosis are considered, even in absence of any visible acute changes, sufficient evidence of AIHD as being a cause of death, 92.3 per cent of autopsied male and 78.8 per cent of autopsied female sudden death cases met up with this requirement. There remained, however 19 cases, 12 male and 7 female cases, in which the sole autopsy finding was coronary arteriosclerosis of moderate or lower grade. The grade of coronary artery stenosis was assessed as being moderate in 13 and as slight in 6 of these. It seems that in these 19 cases, again, additional support for the determined cause of death is called for

A severe chest pain in connection with a fatal attack was reported in three cases. A history of previous myocardial infarction was obtained in one male and in one female case. In another seven male cases a history of angina pectoris during the patient's life was recorded. It may further be mentioned that a history of hypertension was found in four cases while a history of diabetes mellitus was found in none. In three cases a changing pattern of angina pectoris during the preceding month prior to death was reported.

After checking the case histories in those 19 cases mentioned above, there still remained 6 cases (2 males and 4 females) in which neither the autopsy nor the patient's history could be considered to furnish sufficient evidence for diagnosis of AIHD Coronary arteriosclerosis was assessed to be moderate in three and slight in the other three cases. Although other major causes of death were excluded, the cause of death without doubt remained uncertain.

The case histories of the above-mentioned six cases were as follows

1. A 48 year old male who succumbed to momentaneous death without any warning. He had no relatives and no additional information could be obtained.

2. A 61 year old femal who succumbed to momentaneous death whil doing household work. She had not suffered from any atherogenic disease nor complained of any premonitory symptoms.

3. A 63 year old female who succumbed to momentaneous death at home. During the preceding week she had used alcohol in abundance. She had not had any clinical ischaemic heart disease, but there had been congestive heart failure. No information concerning premonitory symptoms was vailable but a physician was known to have called on her merely one day before her death, the reason for the visit, however remaining unknown.

4. A 63 year old female succumbed to momentaneous death in front of her home door. She had not previously had any ischaemic heart disease or other therogenic disease. She had not complained either of any particular symptoms during the last month.

5. A 44 year old female succumbed to momentaneous death at home. No ischaemic heart disease had been diagnosed earlier but she had suffered from dyspnoea of effort. During the last week, she had complained of discomfort in the chest, which was not of the effort angina type, and of tiredness and nausea. She had announced her intention to see doctor for these symptoms.

6. A 65 year old male succumbed to momentaneous death on a beach, after swimming. No history of ischaemic heart disease or premonitory symptoms was vailable. Instead, he had suffered from obliterative arteriosclerosis of the legs, for which a symphatectomy had been performed 8 years earlier

Non-autopsied cases

In 47 cases acute ischaemic heart disease was considered to be the cause of death, without confirmation by autopsy 41 of these were males and 6 were females. Fourteen cases occurred outside Helsinki, two of them abroad. The autopsy rate in the other parts of Finland is much lower than in Helsinki, and this explains why some of the death certificates were signed on fairly feeble grounds.

It is natural that in non-autopsied cases the history of the acute attack becomes most important when the validity of the AIHD diagnosis is considered. Severe chest pain was associated with the fatal attack in 11 male and two female cases. The event was fully momentaneous, without any report of chest pain, in 24 male and four female cases. An atypical attack was reported in three male cases, and in another three male cases there was no accurate information about the attack except for mention of its rapid course.

The prevalence of clinical ischaemic heart disease during life in the non-autopsied cases is presented in Table 8. It seems to be very high among males. No less than 35 out of 41 males had either experienced a myocardial infarction or suffered from angina pectoris during their life. In contrast, only one female had a positive history of previous IHD

It is usually considered that if a person with a known history of ischaemic heart disease meets with sudden death, IHD is also a highly probable cause of death. Such history was lacking in six male and five female cases. In one male and two female cases, however a typical severe chest pain was reported. In another two male cases a

Table 8. *Prevalence of previous myocardial infarction and angina pectoris in non-autopsied cases of sudden coronary death.*

Age	Total	History of myocard. infarction	History of angina pectoris	No history of IHD
		M a l e s		
40—49	4	2	1	1
50—59	13	3	8	2
60—65	24	12	9	3
Total	41	17	18	6
Per cent		41.5	43.9	14.6
		F e m a l e s		
55—59		—	—	2
60—65	4	—	1	3
Total	6	—	1	5
Per cent		—	16.7	83.3

fresh occurrence of angina pectoris during the last month prior to death supported the diagnosis of AIHD The case histories for the remaining 3 male and 3 female cases are as follows

1 A 55 year old man died outside Helsinki, suddenly after swimming. He was single and no relatives or friends could be contacted. No information on previous health or premonitory symptoms was available. The death certificat was signed by a physician at a local hospital, to which the patient was taken after his death.

2. A 63 year old man. The only information available was that death occurred momentaneously within 5 minutes, outside Helsinki. The man was a widower and no relatives could be contacted even the physician who signed the death certificat could not give any additional information.

3. A 65 year old man being treated in a psychiatric hospital died outside the hospital. *There was no history of previous IHD or any* other cardiovascular disease He underwent a rapid attack with pallor and transpiration, which progressed to death in 45 minutes. There was no information bout premonitory symptoms during the last 4 weeks.

4. A 55 year old female died momentaneously outside Helsinki. No information on previous diseases or premonitory symptoms could be obtained.

5. A 61 year old female died momentaneously outside Helsinki She had had hypertension, which was not being treated During the last two weeks she had complained of discomfort in the chest.

6. A 62 year old female died momentaneously in the street. She had earlier been treated for hypertension and congestive heart failure. She had not complained f any prodromal symptoms.

Comments

It is generally accepted that ischaemic heart disease is the commonest cause of sudden death. Therefore in many epidemiological studies, sudden unexpected deaths are considered to be due to AIHD if no other obvious cause of death can be pointed to It must be emphasized, however that autopsy is the only means to demonstrate the cause of sudden death with any certainty

Most community studies in the U.S.A. and Great Britain have reported fairly low autopsy rates. Only 3 out of 45 SCD cases were autopsied in the Tecumseh study (Chiang et al. 1970) The autopsy rate was 30 per cent in Belfast (McNeilly 1967) and 64 per cent in the Edinburgh Community Study (Armstrong et al. 1972). Higher autopsy rates have been reported from Sweden (Sievers 1963 Fodor 1969 Wikland 1971).

A high autopsy rate (80.3 per cent) was arrived at in the present study which should provide a certain guarantee that the majority of these cases truly represent AIHD In fact, the autopsy rate seems to be among the highest reported.

A recent myocardial infarct or a fresh thrombus was found in a high percentage in *spite of the short duration of the attack.* This is in fact strange, although many previous autopsy studies have indicated the same. It can be speculated that the initial phase of the fatal attack has been silent or that the victim has not reported such symptoms as he may have had.

In contrast to the validity of a fresh myocardial infarct, that of acute ischaemic *changes may perhaps be contested. Autolysis* causes similar changes, neither can its role be excluded in this material. On the other hand, changes suggesting acute ischaemia were the only basis for diagnosis in a minority of cases only

It is not unexpected that in many cases no acute changes whatsoever were found at autopsy The percentage of negative post mortem findings should actually be even higher on the basis of present knowledge as to the time needed for the development of visible changes. Scars from old myocardial infarcts and advanced coronary arteriosclerosis were found in high percentage and these at least indicate that the person has really had IHD during his life although the contribution of these findings to the acute fatal event cannot be demonstrated.

The degree to which occlusive coronary arteriosclerosis contributes to sudden death is not established. Some degree is an almost uniform finding in the middle-aged or elderly population and it is not possible to point to any critical degree of occlusion (Roberts 1972).

In the present study it was considered, quite arbitrarily that a severe coronary arteriosclerosis means that the lumen of at least one of the main coronary arteries is totally occluded. This finding was considered to justify the entry of AIHD as a cause of sudden death. In cases with coronary arteriosclerosis of a lower grade, additional support was sought from the case history. This was also obtained in the majority of cases.

After all, there remained only 6 autopsied cases in which the cause of death remained uncertain. However this group only constitutes 3.1 per cent of all autopsied cases and 2.5 per cent of the whole SCD group.

While the diagnosis of sudden coronary death may remain uncertain at autopsy the basis for diagnosis is even weaker in non autopsied cases. Acute chest pain in connection with the fatal attack is considered a strong support for the diagnosis of AIHD but the death is often quite momentaneous without any attendant chest pain. Wikland (1971) has recently shown that 10 per cent of sudden fatal attacks with acute chest pain or dyspnoea were caused by diseases other than IHD.

A high prevalence of previous symptomatic IHD was found in the non-autopsied series. According to the present understanding, when a person with known IHD meets with sudden death, this event is due to AIHD with a high probability rating. There remained only a few cases of momentaneous death without any additional support for the diagnosis.

It must be realized that no general agreement exists concerning the diagnostic criteria of SCD. The cause of death can be determined with a higher or lower probability rating, which varies from case to case. A high autopsy rate, no doubt, improves the level of validity of the diagnosis, but even a hundred per cent autopsy rate would not be able to guarantee correct diagnosis in one-hundred per cent of cases. Against this background the validity of the diagnosis in the present series of SCD can be considered satisfactory.

Summary

192 of the total 239 SCD cases were autopsied (80.3 per cent). The autopsy rate was 79.5 per cent for males and 84.6 per cent for females. This seems to be a very high rate by international standards.

In spite of the short duration of the attack a recent myocardial infarction or a fresh thrombus was found in a high proportion of cases: in 40 per cent of male and 30 per cent of female cases. In 92 per cent of male and 79 per cent of female autopsied cases, the autopsy findings were considered as speaking strongly in favour of AIHD or at least of severe IHD.

The diagnosis of AIHD was supported by previous symptomatic IHD in 36 out of 47 non-autopsied cases.

When all the relevant information was taken into account, the cause of death was considered to be unreliably fixed in a few cases only.

4 DEMOGRAPHIC CHARACTERISTICS

Marital status

The patient's marital status is always entered in the hospital records and also in the death certificates. Therefore, this information could almost invariably be obtained. It is clearly evident from official vital statistics that the distribution by marital status changes markedly with age. In addition to the usual tabulation, it was therefore considered nec

Table 9. Distribution of acut ischaemic heart disease patient _ ferent comparison groups by age sex and marital status.

Age	Sudden deaths Total	S	M	D	W	Non-sudden deaths Total	S	M	D	W	Survivors Total	S	M	D	W	All[1] Total	S	M	D	W
						Males														
—49	43	6	34	3	—	31	2	23	5	1	149	15	117	15	2	234	27	179	25	3
50—59	73	6	56	7	4	72	6	56	7	3	239	15	182	21	14	396	28	306	42	22
60—65	82	8	63	4	8	72	4	55	9	4	148	7	115	12	14	316	20	235	31	30
Total	198	20	153	14	12	175	12	134	21	8	536	34	414	48	30	946	73	720	98	55
Per cent		10	77	7	6		7	77	12	4		6	78	9	6		8	76	10	6
						$\chi^2 = 3.78$ N.S.D.														
						Females														
—49	4	—	2	1	1	5	2	3	—	—	24	3	15	4	2	34	5	20	6	3
50—59	19	1	9	4	5	19	3	8	3	5	79	11	46	8	14	120	16	63	15	26
60—65	16	1	9	3	3	40	12	17	2	9	83	5	38	12	28	158	1	64	18	55
Total	39	2	20	8	9	64	17	28	5	14	186	19	99	24	54	313	43	147	39	84
Per cent		5	51	21	23		27	44	8	22		10	51	12	28		13	47	13	27
						$\chi^2 = 17.22^{**}$														

[1] 52 cases (39 male 13 female) of unwitnessed death included.

S = Single, M = Married, D = Divorced, W = Widowed

essary to investigate the potential association of marital status with sudden death, non sudden death and the survival from an AIHD attack in a manner enabling the effect of age to be eliminated. An age adjustment process was accordingly applied. The method and mathematical treatment are laid out in Chapter III. 4.

Results

Table 9 presents the marital status distribution in the three comparison groups, SCD NSCD and SURV It can be seen that in both sexes a slightly higher number of married persons than expected survived their AIHD attack. Both single and divorced persons were slightly under represented among the survivors. A striking accumulation of single females is found in the NSCD group. The differences were statistically significant among females, but non-significant among males.

Fig 8 presents the three comparison groups, SCD, NSCD and SURV in different marital status categories in the form of age-adjusted observed to-expected ratios. The ratio equals unity if the observed number of cases coincides with the number expected upon age adjustment. The group of un witnessed deaths has been excluded from the figure, although it was included in the calculations. This group had higher numbers than expected in all categories other than that of married persons, and this is fully plausible

The over-all picture of distribution by marital status somewhat changes after age adjustment, as can be found when Table 9 is compared to Fig 8 There are more married people and less single people than expected among the survivors of both sexes. The divorced persons, again, seem to be more numerous than expected among survivors. Sudden death seems to be most often the fate of single males and widowed females The age adjustment abolished the

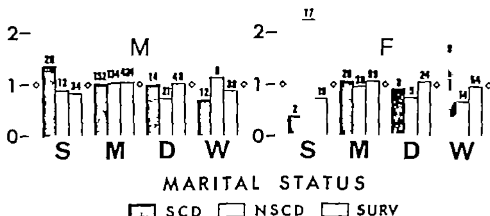


Fig. 8. Preponderance of persons belonging to SCD NSCD and SURV groups, expressed as re-adjusted observed to-expected ratios, in different marital status categories. S = Single, M = Married, D = Divorced, W = Widowed. Figures over each column number of observed cases.

statistical significance which was found among females the differences are now non-significant in both sexes.

Attempts of several kinds were made to account for the effect of the group of un witnessed death. In Fig 8 this group was included in the calculations, although it was not presented in the diagrams. In another analysis this group was excluded and only the actual comparison groups, SCD NSCD and SURV were considered. Finally the unwitnessed death cases were added to the sudden and non-sudden death cases in proportion to the size of these groups. By all methods the result subsequent to age adjust ment was the same the differences in marital status distribution between the SCD NSCD and SURV groups were non-significant.

Comments

Several previous studies have suggested a certain over mortality in IHD among non-married persons giving rise to much specula tion.

A slight trend in the same direction as mentioned above is discernible in the present study It must be realized that in a study of the present kind various bias factors easily cause pseudo-correlations lacking any actual connection with the marital status itself. It is to be fully expected, by definition, that marital status categories other than that of married persons are over represented in the group of unwitnessed deaths. Although this group is relatively small, it still impedes the comparison of the SCD and NSCD groups. A significant bias may be incurred if the effect of age is not taken into account, because the relative proportion of widowed and divorced persons increases strongly in the older age groups.

Finally it has to be emphasized that married persons constitute a great majority of all cases. In Fig 8, the distribution of the comparison groups is most uniform in this largest group. In the other marital status categories, on the other hand, the small numbers may be responsible for seemingly remarkable differences, which turn out to be non-significant.

Summary

After age adjustment no particular marital status category was significantly associated with any one of the comparison groups SCD NSCD or SURV Obvious bias factors were demonstrated and attempts were made to eliminate their influence.

Table 10 Distribution of cute ischaemic heart disease patients in at comparison groups by ge sex and social class (I I IV)

Age	Sudden deaths Total	I	II	III	IV	Non sudden deaths Total	I	II	III	IV	Survivors Total	I	II	III	IV	All Total	I	II	III	IV
								Males												
—49	41	6	10	18	7	31	3	7	14	7	150	32	36	61	20	233	43	56	96	38
50—59	2	14	18	30	10	73	15	16	33	9	239	51	48	107	33	396	81	84	175	58
60—65	52	1	17	37	16	72	18	15	27	12	149	31	22	70	26	316	63	57	141	55
Total	185	32	45	85	33	1 6	36	38	74	28	538	115	106	238	79	945	187	197	413	149
Per cent		16	23	44	17		20	23	42	16		21	20	44	15		20	21	44	16
							χ^2 3 77 N.S.D													
								Females												
—49	4	—	—	3	1	3	—	1	2	—	26	4	1	12	9	34	4	3	18	10
50—59	19	—	5	6	8	19	4	5	7	3	77	3	19	41	14	117	8	29	54	26
60—65	15	—	2	12	1	41	4	5	21	11	93	9	22	37	23	157	13	31	71	42
Total	38	—	7	21	10	63	8	11	30	14	196	16	42	90	48	306	25	63	142	76
Per cent		0	18	56	6		13	17	48	22		8	21	46	24		8	20	46	25
							χ^2=3 94 N S D													

5. ases (39 mal 13 female) of unwitnessed death included.

Social class

The social classification used in Finland is presented in Chapter III. 3. The census data of 1965 for Helsinki reveal that the social class distribution changes markedly with age. It was therefore considered necessary *to* analyse the association of social class with the comparison groups also by means of age-adjusted ratios, as was done in the foregoing concerning marital status.

A quite considerable percentage of all patients with AIHD had retired from work prior to their attack, i.e no less than 31 per cent of males and 37 per cent of females. Therefore, analysis concerning social class was also performed for non retired and retired persons separately

Results

Table 10 presents the social class distribution in the comparison groups, SCD NSCD and SURV Among males the distribution is fairly uniform in all comparison groups. Among females it can be noted that there were no cases *of sudden death in the highest* social class. The differences observed were statistically non-significant.

Fig. 9 presents, in the form of age-adjusted observed to-expected ratios, the distributions of the three comparison groups by four social classes. The diagrams on top refer to those who were still working when the attack occurred in the centre the retired persons have been shown, and at the bottom the *findings concerning both groups together* can be seen.

The picture is seen to be largely the same for all cases on one hand and for working subjects on the other In contrast, the retired persons display a different picture

Among males sudden death is found to be less frequent and survival more frequent than expected in the highest social class I. The opposite is true of the lowest social *class IV The proportion of sudden deaths*

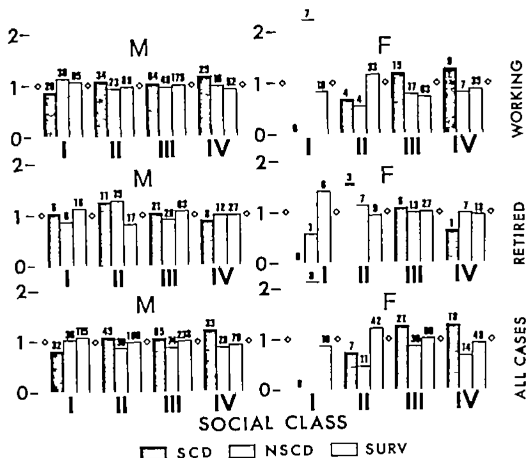


Fig. 9 Preponderance of cases belonging to SCD NSCD and SURV groups, expressed as age-adjusted observed to-expected ratios, in different social classes. Working (top), retired (centre) and all cases (bottom). I = highest, IV = lowest social class. Figures over each column number of observed cases.

appears to increase fairly uniformly from the highest to the lowest social class.

Among females no such lucid picture can be seen. It is notable that there were no sudden death cases in the highest social class. The proportion of sudden deaths actually increases, also among females, from the highest to the lowest social class. The proportion of survivors, however is lower than expected in both social classes I and IV. It should be noted, though, that the ratios are based on small numbers.

Subsequent to age adjustment there were no statistically significant differences among either males or females.

Comments

The association between social class and incidence of IHD has been an object of several studies over the last 20 years (Marks 1967 Antanovsky 1968, Hinkle et al. 1968) The differences observed have usually been at tributed to differences of the average level of physical activity although, without doubt, other differences of importance also exist in the mode of life between different social classes. It seems appropriate to assume that in recent years these differences have diminished and the average on-the-job physical activity commonly considered as explaining

T bl 11 *Prevalence f previous symptomatic ischaemic heart disease (IHD) and myocardial infarction in different comparison groups.*

Age	Sudden deaths Tot	Data av	Myoc Inf	IHD	Per cent	Non-sudden deaths Tot	Data av	Myoc. Inf.	IHD	Per cent	Survivors Tot.	Data av	Myoc. Inf.	IHD	Per cent	All Tot.	Data av	Myoc. Inf.	IHD	Per cent
										Males										
—49	44	37	13	21	57	31	29	13	15	53	150	149	38	78	53	_36	313	64	116	54
50—59	73	68	26	51	77	73	68	34	53	78	239	237	84	184	65	297	372	147	261	70
60—69	83	77	30	61	79	72	66	33	53	80	149	147	54	117	80	320	294	123	237	80
Total	’00	180	69	133		176	163	80	121		538	533	176	350		953	879	334	614	
Per cent			38		74			49		74			33		66			38		70
										Females										
	4	3	1	2	67	4	4	3	3	75	26	26	4	18	69	33	33	7	23	70
	19	16	5	9	56	19	17	3	14	83	76	78	25	54	69	170	112	33	77	69
	6	15	5	6	53	41	39	16	30	77	84	94	29	72	77	150	153	51	112	73
	39	34	11	19		64	60	21	47		198	198	58	144		314	298	91	212	
			33		56			35		8			29		73			31		71

Males: $\chi^2 = 13..8$ (myocardial infarctions); $\chi^2 = 6.96$ (total IHD)

Females: $\chi^2 = 0.74$ N.S.D (myocardial infarctions); $\chi^2 = 5.88$ N.S.D (total IHD)

mal 13 female) of unwitnessed death included.

co of previous IHD

IHD = Myocardial infarction and/or angina pectoris.

the lower incidence of IHD in lower social classes, is continuously decreasing. The role of physical activity will be discussed in detail later.

Data on the relationship between social class and sudden coronary death are scarce. When the results of the present study are considered, the most notable finding is that there were no statistically significant differences between the different social classes. Nevertheless, a slight tendency seems to exist in that sudden death is more frequent in the lower social classes, in which the prognosis seems to be less favourable. This finding, though not significant, corresponds to those studies cited from Helsinki by Gorbatow et al. (1971).

The picture was closely similar in the entire material and in the sub-series of non-retired persons, whereas retired persons, treated as a series of their own, presented a somewhat different picture. This may however be based on small numbers alone. It hardly warrants any conclusions, especially as these differences, too, are non-significant. It is also due to the small numbers that the differences among females, while appearing quite striking in the diagram, do not reach any significant level.

Summary

Social class was not significantly associated with any one of the three comparison groups, SCD, NSCD or SURV. However a slight tendency seemed to exist in that sudden death was more often the fate in the lowest social classes and that it was correspondingly less frequent in the upper classes.

5 PREVALENCE OF CERTAIN PREVIOUS DISEASES AND RISK FACTORS

The same risk factors which predispose to all manifestations of IHD seem to be associated with sudden coronary death, too, although their predictive power may be different in different manifestations of IHD. The symptomatic clinical ischaemic heart disease itself, i.e., a myocardial infarction or angina pectoris, involves a definite excessive *risk of sudden death. It will therefore* also be dealt with in this connection. While many risk factors seem to be independent, the combination of several of them mostly multiplies the risk (Stamler 1967, Epstein 1968). The whole spectrum of risk factors and previous diseases can be observed by methods of multifactorial analysis, as will be presented later. In the following the prevalence of some major diseases and risk factors will be separately analysed.

Symptomatic ischaemic heart disease

The history of myocardial infarction or angina pectoris was based on information gained from the patients or their relatives and, whenever possible, confirmed by hospital records. Both confirmed and non-confirmed episodes of a previous myocardial infarction were accepted. 88 per cent of the episodes could be confirmed, and in this respect there were no significant differences between the groups.

Results

Table 11 presents the prevalence of myocardial infarction and of the total of previously diagnosed *symptomatic ischaemic heart* disease, including both myocardial infarction and angina pectoris.

38 per cent of male and 31 per cent of female patients with AIHD were reported to have had a myocardial infarction in the past. The prevalences were higher for males than for females in all comparison groups and increased with age in both sexes. In both sexes the lowest prevalence was seen in the SURV group and the highest in the NSCD group. The SCD group was intermediate between these. The differences were statistically significant among males but non-

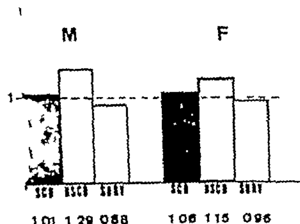


Fig 10 Preponderance of patients with previous myocardial infarction in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

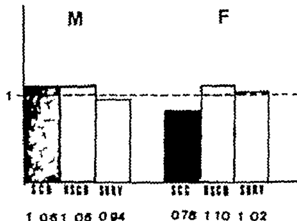


Fig 11 Preponderance of patients with symptomatic ischaemic heart disease (myocardial infarction and/or angina pectoris) in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

significant among females. The distribution of patients with a previous myocardial infarction in the three comparison groups is presented in Fig. 10 as observed-to-expected ratios, separately for males and for females. The accumulation of these patients in the NSCD group is clearly evident.

The sex difference disappears when the prevalence of total symptomatic IHD is considered to be 70 per cent for males and 71 per cent for females with AIHD Among males, the prevalence of symptomatic IHD increased with age whereas that of females remained fairly constant in all age groups. The differences between the comparison groups reached an almost significant level among males. This is due to the lower prevalence in the SURV group compared to the SCD and NSCD groups, in which the prevalences were nearly equal. Among females, again, the prevalence of symptomatic IHD was lowest in the SCD group and highest in the NSCD group but with no significant differences because of the small numbers. The distribution of patients with a diagnosed IHD in the three comparison groups is again presented as observed to-expected ratios in Fig. 11

Comments

It is not unexpected that a great proportion of patients with AIHD in a cross-sectional study already suffer from symptomatic IHD The lower frequency of previous myocardial infarction among survivors, compared to the SCD and NSCD groups, is in accordance with the well known higher mortality in recurrent attacks compared to the first attacks. In both sexes the highest prevalence was found in the NSCD group. This finding too, conforms with several previous studies, which suggest that the mode of death in recurrent attacks is delayed rather than sudden (Achor et al. 1956, Weiss 1956 Liebow and Badger 1963 Hagström et al. 1967 Badger and Liebow 1968, Rissanen et al. 1971).

The prevalence of previous myocardial infarction found in the SCD group seems to be slightly higher than in previous studies, which report prevalences of around 30 per cent (Bainton and Peterson 1963 Fulton et al. 1969) As a conclusion, it can be stated that a history of myocardial infarction seems to increase the risk of death, but the death is more often delayed than sudden.

A somewhat different picture is obtained when the total symptomatic IHD is taken into consideration. Previous studies have reported prevalences of 40 to 59 per cent for total symptomatic IHD among those who experience sudden coronary death (Bainton and Peterson 1963 Myerburg and Davis 1964 Kuller et al. 1966 a, McNeilly 1967 Fulton et al. 1969 Stamler et al. 1969 Chiang et al. 1970 Romo et al. 1971) The prevalence of total IHD in the present series seems very high. This concerns all patients with AIHD In the SCD group, among males, the prevalence of total IHD is as high as 74 per cent among females it is definitely lower namely 56 per cent. In the two other comparison groups the male prominence disappears when the prevalence figures of myocardial infarction and angina pectoris are combined. The prevalence of total IHD is in fact higher for females than for males in the NSCD and SURV groups. This is due to the higher frequency of angina pectoris among females in these groups.

In males, the prevalence figures of total IHD can be interpreted in that a history of IHD increases the risk of dying in an AIHD attack, even though no influence on the mode of death can be seen. Among females the prevalence figures are more difficult to interpret.

Summary

The prevalence of previous myocardial infarction, and that of symptomatic total IHD which includes angina pectoris, is stated in all comparison groups. Among both sexes a history of myocardial infarction was more often associated with death than with survival of an AIHD attack, but the death was more often delayed than sudden. No further conclusion can be drawn from the prevalence figures of total IHD The prevalences of myocardial infarction and total IHD are both higher than those usually reported.

Hypertension

Diagnosis of hypertension was based on the information given by the patients or their relatives and, whenever possible, on hospital records. 89 per cent of all reported instances of hypertension were considered confirmed and the remaining 11 per cent unconfirmed. No significant differences were noted between the comparison groups in the percentages of confirmed and unconfirmed hypertension.

Results

Table 12 shows the prevalence of reported hypertension in the comparison groups. The differences between the groups were almost significant among males but non-significant among females.

The frequency of hypertension appears to be high in both sexes and all ages. It is definitely higher in females than in males and increases with age in both sexes. The only exception is the SCD group of males, where the highest prevalence is found in the age group of 50—59 years.

When the groups with different prognosis are compared, a slight but definite trend can be seen. The prevalence of hypertension is in both sexes lowest in the SURV group, increases in the NSCD group and is highest in the SCD group This trend is more marked among males and only minimal among females. The distribution of patients with reported hypertension in the three comparison groups is presented in Fig 12, as observed-to-expected ratios.

Comments

The high frequency of hypertension in both sexes elicited in the present study parallels those in several previous studies of AIHD Although there are only small differences between groups, a distinct trend of highest prevalence in the SCD group and lowest

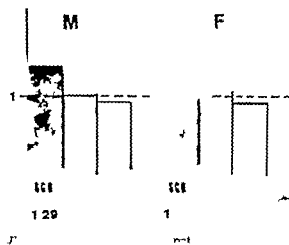
Table 1 Prevalence of hypertension in different comparison groups. [1] 52 cases (39 male, 13 female) of unwitnessed death included.

Age	Sudden deaths				Non-sudden deaths				Survivors				All [1]			
	Total	Data av	Hypertension	Per cent	Total	Data av	Hypertension	Per cent	Total	Data av	Hypertension	Per cent	Total	Data av	Hypertension	Per cent
								Male								
—49	44	35	9	25.7	21	27	3	11.1	150	149	29	19.5	238	214	41	19.2
50—59	4	65	23	35.4	73	65	17	36.2	239	237	47	19.8	397	369	87	23.0
60—65	83	7	22	44	73	63	17	28.2	149	149	39	26.2	320	295	79	26.8
Total	100	177	54	30.5	1 6	157	37	23.6	538	535	115	21.5	953	878	207	23.6
								$\chi^2 = 6.09$								
								Female								
—49	4	3	—	0	4	4	3	75.0	26	26	8	30.8	35	33	11	33.3
50—59	19	15	7	46.7	19	16	6	37.5	78	78	35	44.9	170	111	48	43.2
60—65	16	14	9	64.3	41	34	16	47.1	94	94	45	47.9	159	148	73	49.3
Total	39	32	16	50.0	64	54	25	46.3	198	198	88	44.4	314	29*	132	45.3
								$\chi^2 = 0.32$ N.S.D								

prevalence in the SURV group is noted, which is consistent e.g. with the results of two prominent prospective studies, those of Framingham (Kannel et al. 1971) and Tecumseh (Chiang et al. 1970).

It must be realized that the method used is only a rough estimate of the presence or absence of hypertension. A substantial percentage of hypertensive persons are not aware of their disease. Concepts as to what is considered hypertension, as well as indications for antihypertensive treatment, vary greatly even among physicians. There was also no possibility to take into account the level of blood pressure or the influence of treatment. These obvious drawbacks, common to all retrospective studies impede the evaluation of the importance of hypertension regarding the course of an AIHD attack, in a study of the present kind. It is likely that diagnosed hypertension which fails to be recorded most often occurs in the SCD group. It follows that the association of hypertension with SCD which may be found, is in fact minimized.

An important and possibly significant factor may be, moreover, the role of certain antihypertensive medicines potentially associated with sudden death, independent of the blood pressure level. This especially con



cerns diuretics and the common sequelae of their use, hypokalaemia and disturbances of the heart rhythm. Digitalis and rauwolfia alkaloids, often used together with diuretics, may be speculated as constituting a potentially dangerous combination for some persons at least. In fact, the use of diuretics was more frequent in the SCD group (25 per cent of all these patients) than in the SURV group (12 per cent). The difference was highly significant. However there was no significant difference between the SCD and NSCD groups (24 per cent). The use of digitalis was also more common in the SCD than in the SURV group

The importance of this finding cannot be definitely judged. Its most likely cause is the poorer condition of persons who are subsequently going to die suddenly causing a more frequent use of medicines such as digitalis and diuretics. However the possibility remains that these medicines may play an independent role as causative factors by predisposing to sudden death.

Summary

The prevalence of hypertension on the whole was found to be high in patients with AIHD In both sexes, the prevalence was lowest in the SURV group and highest in the SCD group.

Other cardiovascular diseases

Other cardiovascular diseases, excluding symptomatic ischaemic heart disease and hypertension, constitute in this study a heterogeneous group of diseases or symptoms, which are mostly closely related to atherosclerosis or ischaemic heart disease itself. The diseases comprise e.g. a stroke, and the symptoms include such as congestive heart failure or various disturbances of rhythm, which in fact in most cases are also manifestations of IHD

Results

A stroke has mainly affected persons who already suffered from symptomatic IHD or hypertension. The whole group of AIHD patients contains only 20 persons (16 male and 4 female) with a history of stroke who were reportedly free from the two diseases mentioned. Of them, four males and one female belonged to the SCD group.

35.1 per cent of all patients whose use of medicines was known used digitalis preparations. The corresponding percentages in the SCD NSCD and SURV groups were 38.8 52.2 and 28.9 respectively These differences are highly significant. If use of digitalis is considered an index of heart failure although an unreliable one, it seems as if the heart failure were slightly more frequent in the SCD group and markedly more frequent in the NSCD group than in the whole series of AIHD patients. Therefore, heart failure seems to be associated with death in an AIHD attack, and most often with delayed death. In the majority of cases undoubtedly the basis of heart failure was either IHD itself or hypertension. There were, however 39 patients (27 males and 12 females) with a history of congestive heart failure who were reportedly free from the two diseases mentioned. Of them, 5 males and 2 females belonged to the SCD group This does not mean that these patients would have been free of these diseases, which had not been diagnosed, however

Only a few examples of valvular congenital or pulmonary heart disease were reported as previous diseases. Special attention was paid to aortic stenosis, which is also known to be responsible for sudden fatalities. Only two cases of aortic stenosis were reported in the SCD group, but in both cases autopsy revealed a fresh myocardial infarction, whereby the contribution of the valvular lesion to the sudden deaths in question remained uncertain.

Table 13. *Prevalence of diabetes mellitus in different comparison groups*

Age	Sudden death				Non-sudden death				Survivors				All[1]			
	Total	Data av	Diab mell	Per cent	Total	Data av	Diab mell	Per cent	Total	Data av	Diab. mell.	Per cent	Total	Data av	Diab. mell	Per cent
							Male									
—49	44	36	3	8.3	31	28	4	14.3	150	150	10	6.7	226	218	18	8.3
50—59	73	66	6	9.1	73	68	10	14.7	239	238	25	10.5	397	374	41	11.0
60—65	83	79	8	10.1	72	67	8	11.9	149	149	23	15.4	320	300	41	13.7
Total	200	181	17	9.4	176	163	22	13.5	538	537	58	10.8	953	892	100	11.2
						$\chi^2 = 1.58$ N.S.D										
							Females									
—49	4	3	—	0	4	4	2	50.0	26	26	—	0	33	33	2	6.1
50—59	19	16	1	6.3	19	16	6	37.5	78	78	10	12.8	130	112	17	15.2
60—65	16	15	4	26.7	41	38	12	31.6	94	94	10	10.6	150	153	28	18.3
Total	39	34	5	14.7	64	58	20	34.5	198	198	20	10.1	314	298	47	15.8
						$\chi^2 = 10.80^{**}$										

[1] 52 cases (39 male 13 female) of unwitnessed death included.

Summary

The group of other cardiovascular diseases in the present study represents a heterogeneous group of diseases or symptoms of varying significance. Largest contributions were those of heart failure and stroke, which, however usually displayed concomitant symptomatic IHD or hypertension. The independent contribution of the diseases in this group was small.

Diabetes mellitus

The history of diabetes mellitus was based on information obtained from the patients or their relatives. Confirmation was sought from hospital records. The patients with a positive history of diabetes mellitus present a wide variation in severity and duration of the disease and the treatment which they needed varied from insulin treatment to a restricted diet.

Results

The prevalence of diabetes mellitus in the comparison groups is presented in Table 13. Taking all patients into consideration, the prevalence seems to be somewhat higher among females (15.8 per cent) than among males (11.2 per cent) increasing with age in both sexes.

Among males the differences between groups are small and non-significant. The prevalence was lowest, 9.4 per cent, in the SCD group

Among females the prevalence is definitely highest in the NSCD group (34.5 per cent), and, therefore, the differences between groups among females reach a highly significant level. The prevalences in the SCD and SURV groups were more closely equal, 14.7 and 10.1 per cent, respectively

Comments

The prevalences given in this study for the SCD group, 9.4 per cent for males and 14.7 per cent for females, are on a level with those reported earlier in studies on sudden coronary death. A history of diabetes mellitus was obtained in 11.6 per cent of SCD cases in Baltimore (Kuller et al. 1967). In Tecumseh, eight out of 45 persons (17.7 per cent) who succumbed to sudden death due to AIHD had diabetes mellitus (Chiang et al. 1970). The prevalence of diabetes mellitus was 10.6 per cent among 964 medically unattended death cases in Stockholm. Diabetes seemed to have no association with the duration of the fatal attack (Wikland 1971). In the Framingham as well as the Tecumseh study diabetes mellitus seems to be associated with death in AIHD (Kannel et al. 1967 a, Epstein and Ostrander 1971).

The same trend, though very slight in degree, can be seen in the present study. In both sexes the prevalence of diabetes mellitus was lower among survivors than among all patients with AIHD. On the other hand, death seems to be delayed rather than sudden: the difference noted in females is striking indeed, although the numbers are small. Diabetes mellitus seems to be an unfavourable concomitant disease when an AIHD attack occurs, but it is not a predictor of sudden death. The prevalences in all three comparison groups are higher than those usually reported in populations at large. This, too, is consistent with previous studies and with the well-known atherogenic effect of diabetes mellitus.

Summary

The prevalence of diabetes mellitus was fairly high in all groups of AIHD. A history of diabetes mellitus was more often obtained from persons who died in the AIHD attack, and the highest prevalences were noted in the NSCD group, especially among females.

Patients without any major cardiovascular disease or diabetes mellitus

A certain number of persons with AIHD were obviously free from clinical symptoms of any major disease related to arteriosclerosis and in this respect they could be considered healthy at the moment of their acute attack. Patients with a history of any one of the following diseases were excluded from the consideration: symptomatic IHD, hypertension, stroke, other cardiovascular diseases, and diabetes mellitus. The prevalence of these diseases is presented earlier in this chapter.

Results

Table 14 shows the number of persons reportedly free from the above-mentioned diseases in the comparison groups. Of all patients with AIHD 20 per cent of males, but only 9 per cent of females, were considered healthy. Among males the proportion of healthy persons decreased with age, while among females no clear effect of age could be noticed.

Among males the proportion of healthy persons was greatest in the SURV group (24 per cent) compared to the SCD and NSCD groups in which their proportion was equal (13 per cent). This difference between the male groups reached a highly significant level. Among females the differences between the three comparison groups were non-significant and the proportion of healthy persons was greatest in the SCD group (12 per cent).

The distribution of persons considered healthy in the three comparison groups is presented also in Fig. 13. The accumulation of healthy persons in the SURV group in males is clearly evident in the figure. Among females, the ratios were higher than expected both in the SCD and SURV groups but it must be realized that the number of healthy females was very small indeed.

T ble 14 *Patients without ny majo cardiov cul disea diabetes mellitus in different comparison groups.*

Age	Sudden deaths Total	Sudden deaths D ta a	Sudden deaths Healthy	Sudden deaths Per cent	Non sudden deaths T tal	Non sudden deaths Data av	Non sudden deaths Healthy	Non sudden deaths Per cent	Survivors Total	Survivors Data av	Survivors Healthy	Survivors Per cent	All Total	All Data av	All Healthy	All Per cent
							Males									
—49	44	37	0	4	31	29	9	31	130	149	54	36	236	213	73	34
50 59	3	66	7	11	73	68	4	6	39	237	53	23	397	373	65	17
60 – 65	83	77	7	9	72	66	8	12	149	147	19	13	320	294	35	13
Total	200	180	3	13	176	163	21	13	538	533	176	4	953	879	173	20
							$\chi^2 = 13.31$ **									
							Females									
49	4	3	1	33	4	4	—	0	6	5	—	0	33	33	1	3
50 59	19	16	1	6	19	17	—	0	78	78	13	17	120	112	15	13
60 65	16	15	3	13	41	39	3	8	94	94	7	7	159	153	12	8
T ta l	39	34	4	13	64	60	3	5	196	197	20	10	314	298	8	0
							χ^2 1 73 N.S.D									

52 ses (39 male 13 female) of unwitnessed death included.

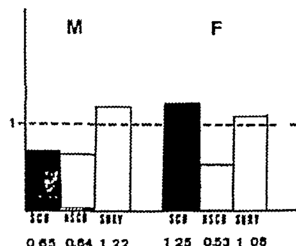
Comments

It is usually thought that AIHD often appears in a previously healthy person without any premonition. On the other hand, several studies have shown that the majority of persons who have an AIHD attack or succumb to sudden cardiac death have suffered before from symptomatic IHD or other diseases related to atherosclerosis.

Relatively few persons with AIHD could be considered healthy prior to the attack in the present study Among males this was even more infrequent among those who died by an AIHD attack. It seems as though healthy persons had a better prognosis than others in the event of an AIHD attack.

Among females only a few persons could be considered healthy prior to the attack and the small numbers hardly warrant any conclusions concerning their distribution in the comparison groups.

In the SCD group only 13 per cent of males and 12 per cent of females were free from any major atherosclerotic disease From the practical point of view this fact might possess great importance It is generally accepted that preventive measures should be



F g 11 Preponderance of patients without any major cardiovascula disease or diabetes mellitus in SCD NSCD and SURV groups expressed as *observed to-expected ratios*

directed to a selected group of persons at the highest risk level. This also applies to the prevention of sudden death. The results presented could be interpreted to signify that directed preventive measures should involve all but a small minority of patients who will succumb to sudden death. However the practical importance of this idea is offset by the high prevalence of the above-mentioned diseases in the middle-aged or in the old.

Ultimately it has to be emphasized that the persons considered healthy in this chapter were in actual fact neither healthy nor free from ischaemic heart disease although for one reason or another they were free from symptoms of IHD or any other major arteriosclerotic disease.

Summary

Only a minority of patients with AIHD 20 per cent of males and 9 per cent of females, could be regarded as being free from any clinical cardiovascular disease or diabetes mellitus prior to the attack.

Among males the seemingly healthy persons seem to have a better prognosis when an AIHD attack occurs. Among females no conclusion whatsoever can be drawn because of the very small number of healthy persons.

Obesity

A body mass index, Quitelet's index calculated as weight per height-squared was used as an index for the patient's degree of obesity. The data were partly based on information gathered from hospital records or gained from the patients or their relatives, partly on measurements made in connection with autopsy

Results

Tabl 15 shows the mean values of body mass index and the standard deviations in the comparison groups.

Tabl 15. *Body mass index of acut ischaemic heart disease patients in different comparison groups, means and standard deviations.*

Age	Sudden deaths				Non-sudden deaths				Survivors				All[1]			
	Total	Data av	Mean	S.D.	Total	Data av	Mean	S.D.	Total	Data av	Mean	S.D	Total	Data av	Mean	S.D.
Males																
—49	44	35	2.612	0.276	31	25	2.465	0.579	150	144	2.596	0.347	236	211	2.561	0.374
50—59	73	60	2.478	0.317	73	62	2.484	0.311	229	220	2.561	0.328	397	362	2.518	0.330
60—65	83	71	2.530	0.380	73	60	2.454	0.331	149	144	2.556	0.356	320	283	2.523	0.338
Total	200	166	2.529	0.330	176	147	2.460	0.374	538	517	2.569	0.341	953	856	2.535	0.351
								F (2,827) = 5.879*								
Females																
—49	4	3	2.469	0.189	4	4	2.229	0.352	26	25	2.423	0.354	35	34	2.395	0.338
50—59	19	17	2.499	0.507	19	16	2.445	0.416	78	74	2.503	0.437	120	108	2.498	0.440
60—65	16	14	2.334	0.563	41	28	2.443	0.533	94	93	2.506	0.366	159	142	2.499	0.444
Total	39	34	2.511	0.502	64	48	2.422	0.478	198	192	2.496	0.393	314	284	2.486	0.431
								F (2,272) = 0.6762 N.S.D								

1 52 cases (39 male, 13 female) of unwitnessed death included.

Tabl 14 *Patient without a y majo cardiovascula diseas or diabetes mellitus in different comparison groups.*

Age	Sudden deaths				Non-sudden deaths				Survivors				All [1]			
	Total	Data a	Healthy	Per cent	Total	Data av	Healthy	Per cent	Total	Data av	Healthy	Per cent	Total	Data av	Healthy	Per cent
								Males								
—49	44	37	9	4	31	29	9	31	150	149	54	36	238	213	73	34
50—59	73	66	7	11	73	68	4	6	239	237	53	22	397	372	65	17
60—65	83	77	7	9	73	66	8	12	149	147	19	13	320	294	35	12
Total	200	180	23	13	1 8	163	1	13	538	533	126	4	953	879	172	20
								$\chi^2 = 15.51$								
								Females								
—49	4	3	1	33	4	4	—	0	26	25	—	0	35	33	1	3
50—59	19	16	1	6	19	17	—	0	78	78	13	17	120	112	15	13
60—65	16	15	2	13	41	39	3	8	94	94	7	7	159	153	12	8
Total	39	34	4	12	64	60	3	5	198	197	20	10	314	298	28	9
								$\chi^2 = 1.75$ N S D								

52 \ses (39 mal 13 female) of unwitnessed death included.

Comments

It is usually thought that AIHD often appears in a previously healthy person without any premonition. On the other hand, several studies have shown that the majority of persons who have an AIHD attack or succumb to sudden cardiac death have suffered before from symptomatic IHD or other diseases related to atherosclerosis.

Relatively few persons with AIHD could be considered healthy prior to the attack in the present study Among males this was even more infrequent among those who died by an AIHD attack. It seems as though healthy persons had a better prognosis than others in the event of an AIHD attack.

Among females only a few persons could be considered healthy prior to the attack and the small numbers hardly warrant any conclusions concerning their distribution in the comparison groups.

In the SCD group only 13 per cent of males and 12 per cent of females were free from any major atherosclerotic disease. From the practical point of view this fact might possess great importance It is generally accepted that preventive measures should be

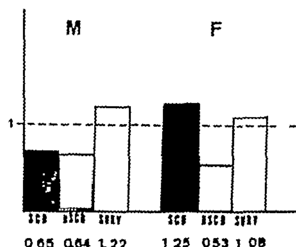


Fig 13 Preponderance of patients without any major cardiovascular disease or diabetes mellitus in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

No clear association between age and body mass index could be found. The lowest means in both sexes appeared in the NSCD group. Among males, the differences between the comparison groups reached a statistically significant level. This was mainly due to the difference between the NSCD and SURV groups. Among females no significant differences appeared.

Comments

No general consensus of opinion exists concerning the demarcation of normal weight and overweight, nor concerning the best index of an individual's obesity although a great range of calculating procedures have been used. A recent study comparing various methods and calculations applied before has led to the conclusion that the body mass index, i.e. weight over height-squared, corresponds best to a person's true obesity provided the basis of estimation consists of weight and height figures (Keys et al. 1972). This index was chosen in the present study.

In a study of this kind, the reliability of the weight and height data is inevitably variable. The figures given by the relatives, or even by the patients themselves, may be wrong. Moreover the data gathered from hospital records do not always reflect the true level of weight in normal circumstances. Autopsy data are not fully comparable with those obtained from other sources. This is an unavoidable methodological shortcoming in a retrospective study.

In the present study the body mass index was definitely lowest in the NSCD group while the means both in the SURV and in the SCD groups were on the same level. Obesity therefore is associated with survival, but if with a fatal attack then rather with sudden death.

Summary

The relative weight of patients with AIHD was determined by calculating a body mass index. The mean values in the SCD group were on the same level as in the SURV group but higher than in the NSCD group. The differences between the groups were significant among males but non-significant among females.

Smoking habit

The history of smoking and, if positive, the daily consumption of cigarettes of every patient were elicited by questioning. The categories with regard to smoking are defined as has been stated before, Chapter III. 2.

Results

Table 16 is a compilation of the smoking habit data in the comparison groups.

Current smokers constituted 66 per cent of all male and 40 per cent of all female patients with AIHD. Half of all female patients, but only ten per cent of all male patients were non-smokers. The relative proportion of current smokers decreased with age in both sexes.

When smoking habits in the different comparison groups are considered, it can be seen that there are only slight differences between the groups among males. The proportion of current smokers was lowest in the SCD group, which correspondingly had the highest percentage of non-smokers. The NSCD and SURV groups showed largely similar distributions within the different smoking categories. The differences were non-significant on the whole.

Somewhat greater differences seem to occur among females, but they too are non-significant owing to the small numbers. In contrast to the males, the proportion of current smokers was highest in the SCD group with the correspondingly lowest percentage of non-smokers.

T ble 1 Di tribution f current smokers among acute ischaemic heart disease patients in different comparison groups by da ly consumption f t bacco

Age	Sudden deaths					Non sudden deaths					Survivors					All 1				
	Tot l	Pipe or ciga	Cigarettes 1 14	15-24	25-	Total	Pipe or cigar	Cigarettes 1 14	15- 4	5-	Total	Pipe or cigar	Cigarettes 1 14	15-24	25-	Total	Pipe or cigar	Cigarettes 1 14	15-24	25-
										Males										
—49	46	—	4	10	13	3	—	7	8	8	117	10	17	57	33	169	10	29	75	55
50—59	43	2	8	15	18	38	—	9	1	8	152	6	50	65	31	234	8	67	103	57
60—65	34	—	14	11	9	37	3	11	13	11	[illegible]	3	27	33	14	149	5	52	58	34
Total	103	2	48	36	39	88	3	27	42	27	345	19	94	154	78	552	23	148	235	146
Per cent		2	25	35	38		3	28	43	28		6	27	45	23		4	27	43	26
Unknown	—					1					3					4				
										$\chi^2 = 13.15$										
										Females										
—49	3	—	1	—	1	3	—	2	1	—	14	—	6	8	—	20	—	10	9	1
50—59	8	—	3	1	4	9	1	5	2	1	39	—	22	13	4	56	1	30	16	9
60—65	3	—	5	—	—	6	—	4	1	1	23	—	15	8	—	35	—	24	10	1
Total	15	—	9	1	5	18	1	11	4	2	76	—	43	29	4	111	1	64	35	11
Per cent		—	60	7	33		6	61	22	11		—	56	38	5		1	58	32	10
Unknown [2]	1					—					—					1				
										$\chi^2 = 11.54$										

10 cases (8 male 2 female) of unwitnessed death with known smoking history included.

[2] Smokers, daily consumption unknown.

In Table 17 the current smokers have been shown according to their daily consumption of tobacco. Pipe and cigar smokers are entered as a separate group cigarette smokers being divided into three classes.

When all patients with AIHD are considered, it appears that male smokers in general consumed more cigarettes per day than females 28 per cent of all male smokers, against only 10 per cent of all female smokers, smoked more than 25 cigarettes daily and were accordingly classified as heavy smokers. The number of pipe or cigar smokers was small, and there were actually none among the females. The frequency of male heavy smokers was somewhat higher in the youngest age group, whereas in females no correlation with age could be noted.

When the different groups are compared, the most marked difference is the accumulation of heavy smokers in the SCD group in males they account for 38 per cent of all smokers in this group, as opposed to 28 and 23 per cent in the NSCD and SURV group respectively The corresponding figures for females were 33 11 and 5 per cent, respectively The differences in both sexes were almost significant.

Fig. 14 presents the distributions of heavy smokers in the three comparison groups. A striking accumulation is seen in the SCD group and the ratio is also higher than 1 in the NSCD group. The same trend is noted among females, with even more marked differences. The observed-to-expected ratio in the SCD group was no less than 4.35

Comments

During the last decade, the relationship between smoking habit and development of IHD has become well established (U.S Public Health Service. Public Health Service publication, No. 1103, 1965 No. 1696, 1967 and No 1696-2. Suppl. 1969 Seltzer 1968, Stamler 1968, Doyle 1969 Seltzer 1970)

In the present study smoking in itself does not seem to influence the course of an AIHD attack. With both sexes the frequency of smoking showed a rather uniform distribution between those who survived their attack and those who succumbed to it. On the whole the prevalence of smoking seems to be very high and higher than in corresponding age groups in the general population of Finland (Kaitaranta et al. 1972)

On the other hand, the accumulation of heavy smokers in the SCD group is seen in both sexes, consistent with previous studies.

Summary

Analysis of the smoking habits of patients with AIHD reveals a high prevalence of cigarette smoking especially in the youngest age group. The frequency of smoking in itself is greatly similar in all comparison groups. Heavy smoking, however seems to be related to sudden death in both sexes.

Physical activity

Data concerning physical activity were lacking at a higher percentage than with regard to most other details of the history This is because only close relatives or other

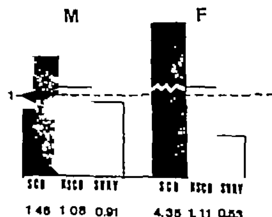


Fig. 14. Preponderance of heavy smokers in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

Table 18 Distribution of ischaemic heart disease patients in different comparison groups by physical activity connected with job

Age	Sudden deaths Total	Data av	R	I	II	III	Non-sudden deaths Total	Data av	R	I	II	III	Survivors Total	Data av	R	I	II	III	All[1] Total	Data av	R	I	II	III
Male																								
—49	44	29	8	10	8	8	31	27	7	6	6	8	150	132	17	48	36	33	235	192	30	64	51	47
50—59	[illegible]	57	16	21	9	11	73	57	19	16	11	11	239	215	38	78	49	50	397	337	76	116	72	73
60—65	83	58	[illegible]	14	11	8	72	57	32	16	3	6	149	135	67	24	23	23	320	[illegible]	[illegible]	54	[illegible]	36
Total	200	144	48	45	[illegible]	25	176	141	58	38	20	25	538	482	122	148	107	105	953	767	233	234	145	156
Per cent			33	31	19	17			41	27	14	18			25	21	22	22			30	31	19	20
							$\chi^2 = 15.4$																	
Female																								
—49	4	3	1	—	—	2	4	4	2	1	1	—	26	21	1	3	7	10	35	29	4	5	8	12
50—59	18	15	5	3	2	5	19	15	6	3	4	—	78	72	17	4	17	14	120	102	30	30	[illegible]	19
60—65	16	14	4	2	7	1	41	34	19	8	7	—	94	84	38	4	17	5	159	137	64	36	31	6
Total	39	32	10	5	9	8	64	53	28	12	12	—	196	177	56	51	41	29	314	268	94	71	63	37
Per cent			31	16	28	25			53	23	23	0			32	29	23	16			37	6	23	14
							$\chi^2 = 19.11$																	

[1] 53 cases (38 male, 15 female) of unwitnessed death included. R = Retired person.

persons very well familiar with the patient's life and habits were able to furnish information reliable enough to enable the physical activity group to be estimated.

Difficulties were caused by the high percentage of retired persons. In the analysis of physical activity linked with occupation, a separate group was formed of these, which was regarded as the group with the lowest activity.

Age adjustment was again applied, in view of the close correlation assumed to exist between age and physical activity.

The criteria for the different activity groups were fixed in advance. It was noted during the study that a great percentage of patients accumulated in the least active group, and only few persons could be assigned to the most active group, in particular in the study of leisure time activity. The original classification was maintained despite this distorted distribution.

Results

Table 18 presents the job-linked physical activity in the comparison groups. Retired persons (R) are shown as a separate zero-group before the least active group I. A considerable percentage of all patients, 30 per cent of males and 37 per cent of females, were retired. This proportion increases strongly with age, of course.

20 per cent of all males belonged to the most active group III. The relative proportion of this most active group was higher than average, 22 per cent, in the SURV group. In both other groups, SCD and NSCD, it was below average: 17 and 18 per cent, respectively. If, on the contrary, the retired persons and activity group I are combined to constitute one least active group, 61 per cent of all males belong to this combined group. It constitutes 56 per cent of the SURV group, as opposed to 61 and 68 per cent of the SCD and NSCD groups, respectively.

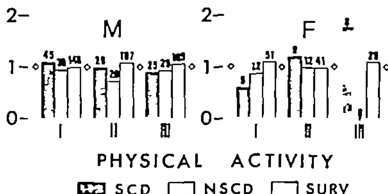


Fig. 15 Preponderance of persons belonging to SCD, NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different classes of physical ctivity at work (I t III) Figures over each column. Number of observed cases.

The differences between the comparison groups were statistically almost significant, but this was mainly due to the different numbers of retired persons in different groups.

When females are considered, somewhat greater differences appear to exist between the comparison groups than among males. The frequency of the most active group III is highest in the SCD group (25 per cent) while the NSCD group contained no females at all belonging to this activity group. If, again, the retired persons and activity group I are combined, 63 per cent of all females belong to this least active group. It constitutes only 47 per cent of the SCD group as opposed to 78 per cent in the NSCD group and 61 per cent in the SURV group The differences between the comparison groups were significant.

Fig. 15 presents the comparison groups as age-adjusted observed-to-expected ratios in three classes of job-connected physical activity Retired persons are excluded. Among males it can be noticed that the SCD cases are represented in excess of expectation in the least active group I. In the most active group III, the contributions of the comparison groups increase from that of SCD to NSCD and to the SURV group

Among females an entirely different pic ture is seen. The least active group I displays uniform increase of contribution in the order SCD—NSCD—SURV as in the male activity group III. In both higher activity groups the proportion of sudden deaths is higher than expected. The differences of the ratios were statistically non-significant in both sexes after age adjustment.

The physical activity connected with lei sure time is presented in Table 19 A strong accumulation in the least active groups can be seen this is particularly notable in the females. Only a few cases were assigned to the activity group III.

Among males, the prevalence distribution appears to be fairly uniform in the comparison groups. When the females are considered, somewhat greater differences seem to be present. This concerns in particular the SURV group on one hand and SCD and NSCD groups on the other The frequency of the least active females was higher among those females who died than among those who survived. However these differences are non-significant owing to small numbers.

Fig. 16 again presents the age-adjusted observed-to-expected ratios in the classes of leisure-time physical activity It must be realized that the most active group III contains very few cases and should be inter

T bl 19 Di tribution f acut ischaemic heart diseas patient different comparison groups by physical activity connected with leisure time

Age	Sudden deaths Total	Data	I	II	III	Non sudden deaths Total	Data a	I	II	III	Survivors Total	Data av	I	II	III	All[1] Total	Data av	I	II	III
									Male											
—49	44	3	14	19	2	31	4	13	9		150	133	63	58	11	238	190	91	84	15
50 59	1	6	7	31	1	73	51	31	3	—	238	217	53	113	12	397	333	153	169	13
60 69	81	64	4	70		7	54	30	3	1	149	129	63	63	1	320	250	139	107	4
T t l	200	158	83	0	5	1 6	132	74	55	3	538	4 9	231	233	4	953	773	383	360	33
P			53	44	3			58	4	2			46	40	5			49	46	4
								χ	6 07 N S D											
									Femal s											
49	4		—	2	—	4	4	1	3	—	16	10	11	5	1	25	16	13	13	1
0 9	19	15	11	4	—	19	15	13	3	—	78	70	46	23	1	120	100	70	29	1
10 9	16	14	13	1	—	41	6	20	6	—	84	82	47	34	1	150	136	83	42	1
Tot l	39	31	4	7	—	64	45	34	11	—	198	172	104	65	3	314	252	183	84	3
P r ent			77	3	—			4	1	—			60	38				68	33	1
								χ	3 79 N S D											

1 es (39 male 13 female) of unwitnessed death included.

preted with great reserve Among males in the activity groups I and II, all three comparison groups appear to be closely equal. However the SURV group is slightly under represented compared to the expected level in the least active group and over-represented in the more active group II and also in the activity group III. SCD and NSCD both surpass the expected level in the least active group and fall below it in the activity group II.

Among females the ratios in the least active group I decrease uniformly in the order SCD — NSCD — SURV The reverse is seen in the more active group II. Only *three females belonged to the most* active group III and they all survived.

The age-adjusted ratios given did not reach a significant level, however either in males or in females.

Comments

Many authors have pointed out that studies of physical activity have so far been hampered by obvious shortcomings and sources of error which are difficult to avoid (Taylor 1967 Altekrose 1968 Paul 1969 Kannel 19 0). The methodological difficulties were also clearly perceived in the present study The validity of the questionnaire remains to be established although an attempt was made to test it, as presented in Chapter III.

The study population represents the whole spectrum of AIHD in the community and a considerable proportion of all patients were previously retired. On the other hand, this implies that the subjects previous health varied greatly and a substantial proportion of them have had to restrict their physical activity due to illness. Cause and effect become mixed.

Occupations which necessitate at least moderate physical activity are becoming fewer all the time (Morris and Gardner 1969). In fact the occupational differences in job-connected physical activity are already now

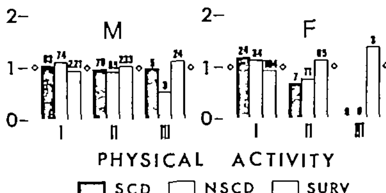


Fig. 18. Preponderance f persons belonging to SCD NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different classes of physical activity during leisure time (I to III). Figures over each column number of observed cases.

so small under urban conditions that highly accurate methods would be needed to show them up. At the same time, the role of leisure time activity is probably increasing in importance. In a middle-aged or older urban population, however habitual physical activity seems to be very infrequent or even spurious.

Finally it must be emphasized that although physical activity may have an independent effect of its own on mortality in IHD its influence is without doubt closely related to many other risk factors. In the foregoing, an attempt was made to eliminate one of them, namely age. Many others, though, remain.

Considering all the reservations made in the foregoing, it is not surprising that only a very slight association between physical activity and the prognosis of an AIHD attack was found. After age adjustment no significant differences remained between the comparison groups.

However the results presented above disclose a slight tendency at least among males, of increasing physical activity being associated with survival more often than with death, though not sudden death in particular. The most prominent finding as regards females was the inverse association found for physical activity on the job on one hand and for leisure time activity on the other. The method used does not allow for any combination of both effects, even though this would be highly desirable. One explanation of the contradictory results among females is certainly furnished by the difficulty of drawing the line between job-connected and leisure time-connected physical activity especially among housewives.

Summary

The job-connected and leisure time-connected physical activity were both determined for patients with AIHD A slight trend was found among males towards more frequent association of survival with high physical activity and correspondingly of death with physical inactivity rather than *vice versa*. There was no clear association with sudden death in particular The same was observed as regards the physical activity of leisure time among females, but consideration of their physical activity connected with the job revealed an inverse association. This may be due to the indefinite demarcation of job and leisure time especially among housewives.

Many methodological difficulties are encountered in a study of the present kind in attempts to determine the potential association of physical activity with the prognosis of an AIHD attack.

Table 70 [illegible] of 13 variables considered in multiple discriminant analysis and their grading.

1	Sex	Male, Female
2	Age	Continuous variable
3	History of myocardial infarction	None, one, more than one
4	angina pectoris	Yes, no
5	stroke	Yes, no
6	diabetes mellitus	Yes, no
7	hypertension	Yes, no
8	Smoking habit	Smoker, Ex-smoker, Non-smoker
9	Daily consumption of cigarettes	0, 1—4, 5—14, 15—25, 25—
10	Body mass index	Continuous variable
11	Physical activity connected with work	I, II, III (Increasing level of physical activity from I to III)
12	Physical activity connected with leisure time	I, II, III (— —)
13	Social class	I, II, III, IV (Decreasing social level from I to IV)

Table [illegible]. Means, standard deviations and F values of the multiple discriminant analysis variables in different comparison groups.

	1 Sex Mean	S D	2 Age Mean	S D	3 Myocardial infarction Mean	S D	4 Angina pectoris Mean	S D	5 Stroke Mean	S D	6 Diabetes mellitus Mean	S D	7 Hypertension Mean	S D
SCD	0.161	0.368	58.44	6.97	0.456	0.736	0.296	0.458	0.865	0.343	0.885	0.319	0.661	0.474
NSCD	0 47	0.433	58. 0	7.15	0.359	0.737	0.276	0.448	0.833	0.374	0.806	0.396	0.707	0.456
SURV	0.261	0.440	54.85	7.33	0.383	0.627	0.347	0.476	0.923	0.263	0.896	0.306	0.732	0.443
F	4.70		6.89		6.63		2.14		8.29		5.58		1.83	
Degrees of freedom	(2, 1058)		(2, 1056)		(2, 1058)		(2, 1031)		(2, 1057)		(2, 1058)		(2, 1049)	

	8 Smoking habit Mean	S D	9 Number of cigarettes Mean	S D	10 Body mass index Mean	S D	11 Physical activity connected with work Mean	S D	12 Physical activity conn. with leisure time Mean	S D	13 Social class Mean	S D
SCD	1.598	0.792	2.229	1.548	2.513	0.359	1.838	0.823	1.479	0.580	2.622	0.928
NSCD	1.625	0 87	2.034	1.500	2.432	0.397	1.753	0.804	1.414	0.528	2.318	0.939
SURV	1.613	0.784	1.989	1.453	2.549	0.358	1.870	0.850	1.546	0.554	2.618	0.974
F	0.06		1.61		6.87		0.90		4.01		0.41	
Degrees of freedom	(2, 1054)		(2, 833)		(2, 1023)		(2, 706)		(2, 1009)		(2, 1058)	

6. MULTIFACTORIAL ANALYSIS OF THE FACTORS RELATED TO THE GROUPS OF SUDDEN DEATHS, NON-SUDDEN DEATHS AND SURVIVORS

In the foregoing, several characteristics and previous diseases of the patients with acute ischaemic heart disease were separately considered in the comparison groups, SCD NSCD and SURV On the other hand, it is well known that the risk factors of IHD are mutually connected in several ways and by no means exert their influence independent of each other

One of the aims of the present study as presented in Chapter I was to test whether it might be possible to differentiate the SCD group from the other comparison groups (NSCD and SURV) on the basis of known risk factors, previous diseases or other characteristics. Therefore, a method was applied which enabled all chosen factors to be taken into account simultaneously The method used was multiple discriminant analysis (Sarna 1970, Cooley and Lohnes 1971).

Results

Thirteen variables were chosen to represent established and suspected risk factors of IHD as well as symptomatic IHD in itself. The variables and their classification are presented in Table 20 If the value of one or two variables was unknown, the mean of the variable or variables in question was substituted for the data lacking. Cases lacking in information regarding more than two variables were excluded from the analysis.

In order to test for differences between the prognostic groups, all variables were tested both separately and in aggregate. The F test was used in the first instance and Wilks' lambda test in the second.

Table 21 shows the mean value for each variable in the three comparison groups, as well as the corresponding F values and their degrees of freedom. It is seen that most of the F values are relatively low implying poor discriminative power of these variables. The value found for Wilks' lambda was 0.911037 and the value for its F approximation was 3.836, with 26 and 2092 degrees of freedom. This reveals that the total discrimination power of the chosen variables was also low

Table 22 presents the thirteen chosen variables in descending order of their reducing effect on Wilks' lambda, after elimination of the effect of intercorrelations. The

Table 22. *Multiple discriminant analysis variables, listed in descending order of their reducing effect on Wilks' lambda*

In brackets, ordinal number of each variable (see Table 20).

Variable		Lambda	F test for additional information
1. Stroke	(5)	0.985	F (2, 2140) = 42.4
2. Body mass index	(10)	0.972	F (4, 2136) = 1 .6
3. Diabetes mellitus	(6)	0.962	F (6, 2132) = 9.8
4. Sex	(1)	0.954	F (8, 2128) = 8.2
5. Age	(2)	0.944	F (10, 2124) = 3.8
6. Daily consumption of cigarettes	(9)	0.936	F (12, 2120) = 2.4
7. Myocardial infarction	(3)	0.928	F (14, 2116) = 1.4
8. Physical activity connected with leisure time	(12)	0.922	F (16, 2112) = 0.8
9. Hypertension	(7)	0.917	F (18, 2108) = 0.4
10. Physical activity connected with work	(11)	0.914	F (20, 2104) = 0.2
11. Smoking habit	(8)	0.912	F (22, 2100) = 0.03
12. Social class	(13)	0.911	F (24, 2096) = 0.005
13. Angina pectoris	(4)	0.911	F (26, 2092) = 0.000

Table 23. Correlation matrix of the multiple discriminant analysis variables. Entries made only where statistical significance exists

* p < 0.05 ** p < 0.01 *** p < 0.001

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Sex													
2. Age	***												
3. Myocardial infarction													
4. Angina pectoris		**											
5 Stroke		**	**										
6. Diabetes mellitus													
7 Hypertension		**			**								
8 Smoking habit	**	**		**			**						
9 Daily consumption of cigarettes			**	**				**					
10 Body mass index			**										
11 Physical activity connected with work		**											
12. Physical activity connected with leisure time													
13 Social clas				**						**			

corresponding lambda value and F approximation are given for each variable The F test was used in this connection to test the additional information which each variable contributes to total discriminant function Because of the mutual correlations of various variables, the order is not the same as indicated by the F values of the same variables in Table 21 For instance age, which has the second highest F value ranges only fifth in this table

Table 23 presents the correlation matrix between the variables Only the significances of the correlations are given.

In order to test in greater detail the two discriminant functions the eigenvalues for both were determined (Table 24) It can be seen that 77.7 per cent of the total discriminant power falls to the share of the first discriminant function, which mainly fulfills the task of separating the survivors from those who died Only 22.3 per cent of the total remained for the second discriminant function, which mainly separates the two death groups, that of sudden and that of non-sudden death.

Table 25 presents the correlations of the variables with the two discriminant functions. The quantitative effect of each variable can be seen. Table 26 shows the mean values for the comparison groups in the two sub-dimensions formed by the two discriminant functions. Finally in Fig 17 the means of each group are presented in the differential space. Tables 25 and 26 together give an overall picture of the differences between the comparison groups.

Table 24 Eigenvalues of the two discriminant functions

Discriminant function	1	0.07438	77.7
Discriminant function	2	0.02143	22.3

Wilks lambda 0.91103
F (16, 2037) 3.836

Table 25. *Correlations between the multiple discriminant analysis variables and the two discriminant functions.*

Variable	Discriminant function 1	2
1. Sex	0.199	0.495
2. Age	—0.428	—0.072
3. Myocardial infarction	—0 410	0.181
4. Angina pectoris	0.242	—0 009
5. Stroke	0 471	—0 054
6 Diabetes mellitus	0.319	—0 400
7 Hypertension	0.168	0.272
8. Smoking habit	—0 006	0.086
9. Daily consumption of cigarettes	—0.163	—0.166
10. Body mass index	0.411	—0.306
11. Physical activity connected with work	0 203	—0.262
12. Physical activity connected with leisure time	0 340	—0 125
13. Social class	0 075	—0.134

It can be found that the male sex exerts a weak effect towards fatal outcome and, in the second discriminant function, a strong influence towards sudden death. Increasing age adds markedly to the chance of dying, but it has only a minimal effect on the mode of death. A positive history of myocardial infarction also increases the possibility of death and has a slight influence favouring non-sudden death. A history of angina pectoris has a slight influence to death, but it is almost without effect on the mode of death. A history of stroke strongly increases mortality but it, too, is devoid of effect on the mode of death.

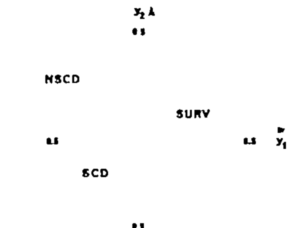


Fig. 17 The comparison groups, SCD, NSCD and SURV plotted in differential space. The total mean vector (2.172, —6 800) was linearly transformed to zero.

Diabetes mellitus favours death and non sudden death, while hypertension promotes death, but primarily sudden death, although this latter influence is relatively weak. Whether any given person is a smoker ex smoker or non-smoker has almost no effect on the prognosis of an AIHD attack. On the contrary if current smokers alone are considered, the heavy smokers have a slight tendency to die and if so, they appear to die suddenly Relative weight exerts a relatively strong influence in the direction of survival and, on the other hand, in the direction of sudden death. The increasing physical activity during work and leisure time both have a slight effect in favour of *survival* and, *in the second discriminant* function, a weak effect in favour of sudden death. Finally social class has virtually no effect at all in either one of the discriminant functions.

Table 26. *Mean val es for th comparison groups in two subd mensions formed by the two discriminant functions.*

Discriminant function	Sudden deaths 1	2	Non-sudden deaths 1	2	Survivors 1	2
Mean	1.894	—7 171	1.724	—6 890	2.370	—6.878
S D	1 110	0 9688	1.058	1 139	0.9495	0 9821

Comments

A multifactorial analysis conveys an overall picture of the significance of the chosen variables which is different, to a certain degree, from that obtained by the usual observation method. All relevant variables may be considered in a quantitative manner both individually and as intercorrelated variables. Those variables yielding no additional information can be excluded. The direction of the effect of each variable is discernible even if it is minimal. The method used is accurate in contrast to the information gathered, which unfortunately was too vague and unreliable to allow for efficient discrimination.

Unavoidable drawbacks are associated with a study of the present kind, in which information is gathered in a retrospective manner. The history of previous diseases and habits is not very reliable and the validity of information varies in different comparison groups. Most of the survivors were personally interviewed, as were some of the patients in the non-sudden death group. Case records from the hospital, at least, were almost invariably available. In contrast, the history of persons who died suddenly was almost exclusively based on interviews of their spouses or other relatives. Owing to the small differences between the comparison groups the significance of information lacking became very marked. This was clearly experienced in the attempts to find the most appropriate manner of dealing with unknown data.

Retrospective studies also preclude quantitative investigation of previous diseases in that often only a dichotomic classification [illegible] feasible. Compared with a prospective study this is an obvious drawback in the case of variables such as hypertension. The importance is further accentuated if one considers that a substantial percentage of persons are unaware of the[illegible] hypertension.

Only two variables could be analyzed as continuous variables, namely age and relative weight. As for the other variables merely an arbitrary classification could be set up. The classification of dichotomic variables is rough, but at all events distinct. On the other hand variables such as smoking habit and social class had to be divided into classes without any assurance of their linearity and appropriate scale.

The list of predominance of the variables, presented in Table 22, is surprising to a certain extent. It appeared that the history of stroke had the best discriminant power of all variables chosen, after the intercorrelations between the variables had been eliminated. A positive history of stroke is strongly associated with death, but the mode of death was more often that of delayed death. In this connection a previous stroke can be considered an expression of advanced arteriosclerosis. It must be emphasized, however that the discriminant power of all chosen variables was relatively low and, therefore the order of the variables is of less importance.

The prevalence of different previous diseases and risk factors in the comparison groups has been considered separately before. It was clearly evident that the differences were very small indeed on the whole. Therefore it is not unexpected that multiple discriminant analysis was also unable to separate the groups very well. It appears that it is hardly possible, on the basis of the patient's history only, to predict in advance which persons are going to die when an AIHD attack occurs and even less which ones will die suddenly. Prospective studies are capable of producing more reliable information like the results of clinical examination, laboratory tests and electrocardiograms, for instance. It is, therefore believed that the outlook as regards predicting the fate of individual persons may also be rather different in their connection.

Summary

A multiple discriminant analysis was applied in order to test the possibility of separating the three comparison groups, i.e. the SCD NSCD and SURV groups, on the basis of the history of previous diseases and other risk factors. Thirteen variables were chosen for the analysis. Unfortunately the discrimination power of the variables was relatively low 77.7 per cent of the total discriminant function falls to the first discriminant function which separates the survivors from those who died. Only 22.3 per cent of the total remained for the second discriminant function which separates the SCD and NSCD groups.

Factors which add to the chance of dying are the male sex, increasing age, a positive history of myocardial infarction, angina pectoris, stroke, hypertension or diabetes mellitus, and low physical activity In the second discriminant function especially male sex had a strong influence in the direction of sudden death. The heavy smokers have also a tendency to die suddenly Increasing relative weight has an influence in the direction of survival and, on the other hand, in the direction of sudden death. Social class had no effect at all.

The available, comparatively unreliable information on the above-mentioned factors seemed to offer no opportunity for pointing out in advance, with sufficient accuracy the persons at the highest level of risk of dying suddenly

7 PREMONITORY SYMPTOMS

A query was made concerning premonitory symptoms during the last four weeks before the AIHD attack in all cases of the present study The following symptoms in particular were inquired after angina pectoris of recent onset r a change in pattern of previous angina pectoris, discomfort in the chest, heaviness of the arms, unusual dyspnoea, unusual tiredness or first occurrence of palpitation. Only those symptoms which really had appeared or had changed their character within this period were accepted as premonitory symptoms.

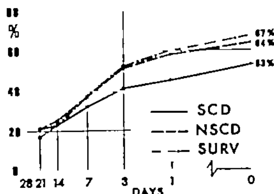


Fig 18. Cumulative graphs showing occurrence of premonitory symptoms during 28 days prior to acute ischaemi heart disease ttack in the SCD NSCD and SURV groups.

Results

A high frequency of prodromal symptoms was reported in all three comparison groups. The frequency was highest in the SURV group (67 per cent) followed by the NSCD group (64 per cent) and lowest in the SCD group (53 per cent) The given percentages refer to cases in which the history of symptoms during the preceding month was available and in which one or several of the symptoms were reported. The differences were statistically almost significant.

Fig 18 shows the times at which premonitory symptoms occurred in the comparison groups, in the form of cumulative prevalence graphs. All three graphs run closely parallel with each other although that of the SCD group stays somewhat lower at the end. According to the figure, the frequency of prodromal symptoms appearing during the fourth week before the attack is fairly high in all groups during the two consecutive weeks it remains lower and an abrupt rise once more occurs during the last week.

Fig. 19 presents the frequency of premonitory symptoms in all three comparison groups. The percentages given refer to the total number of patients in each subgroup. The frequency of those symptoms which were considered most important, namely angina pectoris and unusual dyspnoea, is also presented. It can be seen that the frequency of reported angina pectoris was definitely higher in the SURV group (35 per cent) than in the NSCD (22 per cent) and SCD groups (16 per cent). These differences were statistically highly significant. The contribution of unusual dyspnoea was less and differences between the comparison groups were also small.

The frequency of those persons who were reportedly without any prodromal symptoms was on the same level in all subgroups. In contrast, the amount of unknown data was substantially higher in both groups of dead subjects, as opposed to the SURV group.

Table 27 states the frequency of visits to a doctor over the same period of four weeks preceding the acute attack. It has been found that more than half of all patients in the SCD and NSCD groups had seen a doctor during this period. Only minimal differences exist between these two groups. Visits to a doctor were far more infrequent in the SURV group. Compared with the SCD group, the difference was highly significant for males and almost significant for females. At the bottom of Table 27 the relative number of those visits has been stated which were made on account of premonitory symptoms. It can be seen that in the SURV group consulting a doctor for premonitory symptoms was relatively more common especially among males, differing almost significantly from the SCD group.

Comments

Herrick (191) had al d noticed that a symptomatic phase o ten preceded a myocardial infarction. In 19 F l as w ll as

* Those with Angina Pectoris excluded

Fig. 19 Prevalence of premonitory symptoms in the SCD, NSCD and SURV groups.

Sampson and Elliaser estimated the frequency of premonitory symptoms before a heart attack, which they found to be 50 and 48 per cent respectively. Recent studies of hospital patients with a myocardial infarction have demonstrated even higher rates: Solomon et al. (1969) 65 %, Stowers and Short (1970) 68 %, and Hochberg (1971) 84 %.

It has been more difficult to gain reliable information on premonitory symptoms of sudden death. Adelson and Hoffman (1961) in a study consisting of 500 consecutive sudden deaths defined as occurring within two hours, noticed prodromal symptoms in 49 per cent of cases. Bainton and Peterson (1963) reported that prodromal symptoms occurred in 59 per cent of fatal cases of AIHD. In a Danish study consisting of autopsied sudden coronary deaths at a young age 45 per cent of cases presented prodromal symptoms more than 24 hours prior to death (Hansen 1968). In Oxford, England, Kinlen (1969) noted a recent onset or change in pattern of pain in the chest or epigastrium during the last month before the heart attack, in 49 per cent of patients with a

Table 27 Visits to a doctor of acute ischaemic heart disease patients during four weeks preceding the attack.

	Sudden deaths				Non-sudden deaths				Survivors			
	Males		Females		Males		Females		Males		Females	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Total	200		39		176		64		538		198	
Data available	170		32		165		56		529		194	
Time prior to attack												
—1 week	39	23	10	31	48	30	16	29	78	15	39	20
1—2 weeks	22	13	4	13	12	8	3	5	39	7	20	10
2—4 weeks	29	17	2	6	28	18	11	20	58	11	25	13
Total visits	90	53	16	50	88	56	30	54	175	33	84	43
Visits caused by premonitory symptoms	30	33	7	44	39	45	10	33	82	47	36	43

myocardial infarction, and in 33 per cent of patients who died suddenly

The frequency of all reported symptoms in the present study seems to be on the same level as in the above-mentioned studies. It is possible that the lower frequency noted in the SCD group compared to the NSCD and SURV groups, is merely due to the fact that the history of patients who die suddenly is usually less accurately known than that of patients in the other groups. This perhaps concerns rather more details such as premonitory symptoms than, for example, the history of previous diseases, of which the relatives probably are more often aware.

An obvious shortcoming, and in fact serious limitation, of all above-mentioned studies, including the present one, is their retrospective nature. Preliminary results from a prospective study on unstable angina have lately been reported from Edinburgh, and they convey a somewhat different picture (Fulton et al. 1972). Although registered unstable angina progressed to a myocardial infarction in 14 per cent of cases, sudden death was an infrequent sequela.

A change in angina pectoris is usually considered the most important symptom indicating a potential risk, but its frequency in the present study is fairly low in the SCD group On the other hand, it is impossible to presume that all patients who complained of indefinite symptoms like tiredness could have been taken under close medical surveillance. Therefore it seems unlikely that persons experiencing SCD could be separated from persons in whom the course of an AIHD attack is less severe, on the basis of prodromal symptoms.

More than half of all patients who died in an AIHD attack had visited a doctor during the preceding month, and more than one-third of these visits were caused by prodromal symptoms. The frequency seems surprisingly high, but similar frequencies have also been reported earlier 40 per cent of victims from sudden death in Edinburgh (Fulton et al. 1972), and 50 per cent in Oxford (Kinlen 1969) had seen their doctor within the preceding month. In Oxford 25 per cent, and in Baltimore (Kuller et al. 1966 a) 24 per cent, of patients who died suddenly had visited a doctor during the preceding week. The corresponding figure for the last week was 24 per cent in the present study

It is conceivable that a high frequency of visits to a doctor might offer an opportunity to take the patients at risk under medical control at the most appropriate phase. On the other hand, the greater part of the visits were for reasons other than premonitory symptoms of an AIHD attack. The character of the premonitory symptoms is mostly so indefinite that it is hardly to be expected that their serious nature would be correctly interpreted by the patient or his doctor. It is however worth while to note that roughly 18 per cent of all patients who died suddenly paid enough attention to their premonitory symptoms to make them see their doctor for them.

Summary

53 per cent of patients in the SCD group were reported to have had some premonitory symptoms during the preceding four weeks before the attack, and 16 per cent experienced a change in their angina pectoris symptoms. The corresponding figures were somewhat higher in the other groups.

More than a half of the patients who succumbed to sudden death saw their doctor during the preceding four weeks, and 24 per cent during the last week. More than one-third of the visits were caused by premonitory symptoms.

8 ATTENDANT CIRCUMSTANCES OF THE ACUTE ATTACK

Accurate knowledge of the circumstances in which the sudden coronary fatalities occur also seems necessary before any control programme can be planned. The place and time of occurrence, the activity at onset of symptoms as well as the steps taken to obtain medical help were considered essential points which require clarification. Since in general the fatal attacks were witnessed, this information was in fact obtained in a great majority of cases.

Table 28 *Distribution of sudden death cases by sex and time of day of onset of attack.*

Time of day hrs	Males	Females	Total
00—06	38	6	44
06—12	53	10	63
12—18	55	9	64
18—24	54	14	68
Total	200	39	239

$\chi^2 = 3.88$ N.S.D (Males) $\chi^2 = 3.28$ N.S.D (Females)

Results

Table 28 presents the time of occurrence of the fatal attack in sudden death cases. The attacks seem to occur quite evenly throughout the 24-hour day except during the first six hours, from 12 p.m. to 6 a.m., when definitely fewer attacks occurred than were expected.

Table 29 shows the activity in which the patients were engaged when their acute attack occurred. It can be seen that the activity was rather closely similar in all three comparison groups. The majority of the attacks occurred at rest. Strenuous activity was only rarely reported, more often among males (in 6 per cent) than among females (in 2.5 per cent). Eight entries of strenuous activities in the SCD group consist of four patients engaged in sport, three who were mounting stairs and one who was carrying a heavy object when death occurred.

In addition to the activity at the exact moment of occurrence of the acute attack symptoms, the relatives were further asked concerning any extraordinary stress experienced by the victim immediately before the attack. Various kinds of extra strain were reported. The reports in the SCD group include 2[illegible] cases of exceptional physical stress (9 per cent of all SCD cases). The reported activities mainly consisted of sport activities or of heavy household work, such as shovelling snow, etc.

Table 29. *Distribution of acute ischaemic heart disease patients in different comparison groups by activity at onset of attack.*

Activity	Sudden deaths		Non-sudden deaths		Survivors		Total	Per
	No.	Per cent	No.	Per cent	No.	Per cent	No.	cent
Males								
Rest	118	63.4	90	62.9	295	58.1	516	60.6
Work	61	32.8	44	30.8	1 8	35.0	285	33.5
Strenuous activity	7	3.7	9	6.3	35	6.9	51	6.0
Total, activity known	186	100.0	143	100.0	508	100.0	852	100.0
Activity unknown	14		33		30		101	
Female								
Rest	20	55.6	33	63.5	116	63.4	175	62.9
Work	15	41.6	18	34.6	62	33.9	96	34.5
Strenuous activity	1	2.8	1	1.9	5	2.7	7	2.5
Total, activity known	36	100.0	52	100.0	183	100.0	278	100.0
Activity unknown	3		12		15		36	

Twelve cases of exceptional mental stress were reported, in two cases a severe quarrel had shortly preceded the patient's death. Five patients bathed in sauna shortly before the attack. In seven cases, the use of alcohol preceded death.

Table 30 presents the places of onset of symptoms in the three comparison groups. It can be seen that the majority of attacks occurred at home, more frequently among females (69 per cent) than among males (58 per cent), and more frequently among survi

Table 30. *Distribution of acute ischaemic heart disease patients in different comparison groups by place of onset of attack.*

Place of onset	Sudden deaths		Non-sudden deaths		Survivors		Total	Per
	No.	Per cent	No.	Per cent	No.	Per cent	No.	cent
Males								
Work place	13	6	18	11	66	12	97	10
Home	95	48	76	46	341	64	536	58
Other place	67	34	33	20	93	17	199	21
Hospital	25	12	12	7	14	3	51	5
Onset indefinite	—	—	27	16	20	4	49	5
Total known	200	100	166	100	534	100	932	100
Unknown	—		10		4		21	
Females								
Work place	1	3	2	3	9	5	12	4
Home	22	56	40	65	142	72	212	69
Other place	13	33	5	8	25	13	43	14
Hospital	3	8	11	18	10	5	25	8
Onset indefinite	—	—	4	6	11	6	15	5
Total known	39	100	62	100	197	100	307	100
Unknown	—		2		1		7	

Table 31. *Distribution of acute ischaemic heart disease patients in different comparison groups by place of death.*

Place of death	Sudden deaths No.	Per cent	Unwitnessed deaths No.	Per cent	Non-sudden deaths No.	Per cent	Total No.	Per cent
Males								
Work place	12	6	—	—	—	—	12	3
Home	85	43	26	68	22	13	133	32
Other place	64	32	12	31	15	9	90	22
Hospital	25	13	1	2	127	73	153	37
During transport	11	6	—	—	4	2	15	4
Outpatient department	3	2	—	—	8	3	9	2
Total known	200	100	39	100	174	100	413	100
Unknown	—		—		2		2	
Females								
Work place	1	3	—	—	—	—	1	1
Home	21	54	9	75	4	6	34	30
Other place	12	31	2	17	1		15	13
Hospital	3	8	1	8	56	88	60	52
During transport		5	—	—	2	3	4	3
Outpatient department	—	—	—	—	1	2	1	1
Total known	39	100	12	100	64	100	115	100
Unknown	—		1		—		1	

vors than among those who died. Home was also the commonest place of occurrence in the SCD group although, on the other hand, in this group the category »other place» was proportionally more strongly represented than in the other two groups. 5 per cent of males' and 8 per cent of females AIHD attacks were experienced by persons already hospitalized for various other reasons.

Table 31 similarly shows the places of death. In addition to the SCD and NSCD groups, the unwitnessed deaths were also included in the table. Deaths in transit to hospital and deaths at the outpatient department of a hospital within one hour of arrival, have been distinguished as separate categories because of the practical importance of this information. Both categories remained small: only 4 per cent of all AIHD deaths occurred in transit and per cent immediately after arrival in hospital.

The efforts made to transport to a hospital those patients who ultimately fell into the SCD group have been detailed in Fig. 20. The initial grouping of these patients is according to the place of onset of their symptoms. The diagram reveals that a great majority of the patients died at the same

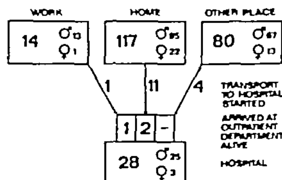


Fig 20. The f t f the SCD cases, presented according to the place of onset f symptoms with particula reference to fforts made to transport th patient t hospital

place before any transport was available. 16 patients only were probably still alive when their journey to the hospital commenced. Ultimately only three patients arrived alive at the outpatient department, while the rest had all died in transit.

In only four other cases the ambulance was summoned while the patient was still alive, but it did not reach the patient before he died. In two further cases a physician on duty was called while the patient was still conscious, and he reached the patient prior to death in one of the two cases.

Comments

The exceptionally small number of sudden deaths during the night hours is obviously due to the fact that many such deaths remained unwitnessed and fell into the "unwitnessed death" category rather than into the SCD group. Therefore, the sudden death cases are not thought to be associated with any particular time of day.

The majority of deaths seem to have occurred while the patient was sleeping or resting, while association of death with strenuous activity was rare. This is in agreement with several previous studies (Babson and Helpern 1948, Spain and Bradess 1957, Weinberg and Helpern 1959, Adelson 1961, Spiekerman et al. 1962, Sievers 1963, Pell and D'Alonzo 1964, Spain 1964, Kuller 1966, Kuller et al. 1966 a, Hansen 1968, Wikland 1968, Lindström 1969, Moss et al. 1969, Solomon et al. 1969, Paul and Schatz 1971, Wikland 1971). Differing from the general consensus, Yater et al. (1948) found that acute attacks of young persons occurred more often during heavy physical activity than could be expected.

It is naturally very difficult or even impossible to assess the true frequency of strenuous activity in the population at large. The results, therefore, justify only the conclusion that strenuous activity probably does not significantly influence the course and termination of an AIHD attack when it occurs. The higher frequency of strenuous activity among males compared with females obviously merely reflects the males' more frequent pursuit of such activities. Finally it must be stated that the strenuous activities reported cannot by any means be considered extraordinarily heavy for a healthy person.

It is possible, on the other hand, that extra strain before the attack could be more important than that undergone at the very moment when the first symptoms of an AIHD attack appear. The consideration may further be extended to comprise not only physical stress but also unusual mental stress. The time interval during which such factors can be assumed to exert an influence is not known.

The frequency of extra strain during the period immediately prior to sudden fatality was higher than at the onset of the attack, but the inquiry also concerned a period of two to three hours. Reported mental stress mainly consisted of various kinds of daily trouble. In two cases a severe quarrel preceded death. The reported sauna episodes and indulgence in alcohol can hardly be considered anything but spurious.

A closer analysis of all reported preceding stresses reveals that the great majority can hardly be considered unusual. It is also probable that the relatives tend to overemphasize the importance and the degree of preceding strains.

The distribution according to the place of onset of symptoms found here resembles those in previously reported studies. It is only natural that the most common place should be the home since the patient spends the greater part of his time at home, especially the females.

Several studies have shown that approximately two thirds of all IHD deaths occur out of hospital. Of course this percentage lies at a much higher if only cases [illegible] ending fatally within one hour are taken

all aid comes too late and the patient is lost if nothing is done before medical aid is obtained. Four minutes is the critical time interval in cardiac arrest if circulation and respiration is not maintained. In theory it might be possible to teach resuscitation procedures to laymen, in particular to the relatives of patients with known IHD. It has also been suggested that patients who have symptomatic IHD should be provided with self-administered medicines to enable them or their relatives to give them a rhythm-stabilizing injection at once if an attack occurs (Levine 1969). However it is hardly realistic to assume that the measures proposed could significantly decrease the acute mortality of AIHD attacks.

Not even prodromal symptoms seem to distinguish the victims of sudden death from persons with a more favourable course for their attack. It has been proposed that special pre-coronary care stations should be established in order to observe those patients who display alarming symptoms, such as a change in the pattern of angina pectoris (Lown et al. 1969 a). On the other hand a recent report from Edinburgh (Fulton et al. 19??) seems to indicate that unstable angina pectoris is no good predictor of subsequent sudden death.

Although various measures can be discussed speculatively (Sidel et al. 1969, Yu 197?), the fact remains that the possibilities of intervention of any kind either at the onset of the attack or during the prodromal phase seem to be limited to those cases characterized by the most acute course. There remains the possibility of prevention, but as has been mentioned earlier the immediate risk group cannot be identified with sufficient accuracy. At present, the natural risk group consists of persons who already have symptoms of ischaemic heart disease. Two-thirds of the persons dying suddenly in the present study had suffered from a symptomatic IHD before their death.

All preventive measures, whether primary or secondary, which decrease the incidence of AIHD attacks probably also decrease the incidence of sudden coronary death. Unfortunately, at present, too little is known concerning the most suitable methods and concerning the effects of any large-scaled programme.

When prevention of sudden coronary death is considered, the basic question is, however, how to prevent fatal disturbances of the heart rhythm. Numerous antiarrhythmic medicines are available. Unfortunately none of them seems to be suitable for long term prophylactic use in large populations. A suitable medicine should be both effective and safe. For example, procainamide and quinidine, though often effective, contain a potential risk of dangerous side-effects. Too little is known in this respect about the beta blocking agents. Quite recently clofibrate has given promising results in decreasing the sudden coronary death rate (Ischaemic Heart Disease. A secondary prevention trial using clofibrate 1971 and Trial of clofibrate in the treatment of ischaemic heart disease 1971).

GENERAL SUMMARY

During one year 1970 1267 cases of acute ischaemic heart disease, 953 males and 314 females, fulfilling the minimum criteria of WHO were registered in the Helsinki population aged 65 years or younger The case fatality rate was 43.6 per cent for males and 37.1 per cent for females. 239 cases (18.8 per cent of all AIHD cases) represented sudden coronary death cases, defined as deaths occurring within one hour from the onset of symptoms. These cases and their characteristics were the actual object of the present study Cases with a different out come, i.e. non-sudden death or survival constituted the comparison groups.

Deaths within one hour constituted a remarkable proportion of all AIHD deaths, 53.1 per cent of all male and 38.5 per cent of all female deaths with known duration of the fatal attack. At the same time these figures reveal a marked sex difference in the course of an AIHD attack, sudden death being far more often the fate of males than of females. The incidence of sudden coronary death increases strongly with age in both sexes, but its relative proportion of all AIHD cases is rather closely similar in different age groups. The incidence seems to be high, although figures of an equivalent level have been reported earlier from other countries having a high IHD mortality in general.

The prevalences of certain demographic characteristics, previous diseases and established risk factors were determined in the comparison groups. The aim of this part of the study was to find those characteristics, if any which might help to identify in advance those in great risk of dying suddenly

It was found that the above-mentioned comparison groups were very similar with regard to most of the factors observed. After age adjustment no particular marital status category or social class was significantly associated with any one of the comparison groups, SCD NSCD or SURV A history of previous myocardial infarction was more often associated with death than with survival of an AIHD attack, but the death was more often delayed than sudden. The prevalence of hypertension was highest in the SCD group and lowest in the SURV group The highest prevalence of diabetes mellitus was found in the NSCD group. Only 20 per cent of males and 9 per cent of females could be regarded as being free from any clinical cardiovascular disease or diabetes mellitus prior to the attack. These persons had a better prognosis when an AIHD attack occurred. The body mass index was lowest in the NSCD group and on the same level in the other comparison groups, SCD and SURV The frequency of smoking was very similar in all comparison groups. On the other hand, heavy smoking seemed to be related to sudden death. Physical activity seemed to have a slight influence favouring survival.

An attempt was made to separate the three comparison groups, SCD NSCD and SURV by the aid of multifactorial analysis, i.e. multiple discriminant analysis. Although this method enables all selected factors to be considered simultaneously both together and separately in a quantitative manner the

end result was that on the basis of the information collected it was not possible to predict very well which persons' AIHD attacks would terminate in sudden death.

The analysis of premonitory symptoms revealed that a high percentage of all AIHD patients had complained of some symptoms during the preceding month. In the group of sudden deaths their prevalence was lowest, but the difference was small. The frequency of change in the pattern of angina pectoris, regarded as the only symptom which could be considered specific in some degree was also lowest in the group of sudden deaths.

The place of onset of symptoms was most often the home just as it was in the comparison groups. Most attacks occurred at rest and those in connection with strenuous activity were very rare. The great majority of patients whose ultimate fate was sudden death died at the same place where the attack began and only a few patients could be brought under adequate medical care. This was obviously due to the unfavourable duration of the attack, not to the inadequacy of the emergency system.

It was noted that both the occurrence of prodromal symptoms and the circumstances surrounding the acute attack were greatly similar in the comparison groups, and did not substantially contribute to differentiating the sudden death cases.

A high autopsy rate was achieved altogether 80.3 per cent of sudden coronary death cases, and the autopsy finding of recent myocardial infarction was surprisingly high. In a high percentage of the non-autopsied cases, the defined cause of death was supported by a previous symptomatic ischaemic heart disease. The cause of death remained uncertain in a few cases only.

In the general discussion the possibilities of preventing sudden coronary death and those of intervention with the aim of decreasing the number of sudden fatalities are discussed on the basis of the findings made in this study. Identification of the persons at the greatest risk of dying suddenly appeared to be difficult. It is also far from established what should be done for these persons if they could be identified.

REFERENCES

Achor R. W P Futch, W D Burchell, H. B. & Edwards, J E. The fate of patients surviving acute myocardial infarction. Arch. Intern. Med. 98 162, 1956.

Adelson, L. Sudden death from coronary disease — the cardiac conundrum. Postgrad. Med. 30 129 1961.

Adelson, L. & Hoffman, W.. Sudden death from coronary disease. Related to lethal mechanism arising independently of vascular occlusion or myocardial damage. J Amer med. Ass. 176 129 1961.

Adgey A. A. J Allen, J D Geddes, J S James, R. G. G Webb, S. W Zaidi, S. A. & Pantridge, J F Acute phase of myocardial infarction. Lancet 2 501 1971

Adgey A. A. J Nelson, P G Scott, M. E Geddes, J S., Allen, J D Zaidi, S. A. & Pantridge, J F Management of ventricular fibrillation outside hospital. Lancet 1 1169 1969

Albrink, M. J & Man, E. B Serum triglycerides in coronary artery disease. Arch. intern. Med. 103 4, 1959.

Altekruse, J M. Exercise and cardiovascular conditioning. J occup. Med. 10 296, 1968

Anderson, T W Le Riche, W H. & MacKay J S. Sudden death and ischemic heart disease. Correlation with hardness f local water supply New Engl. J Med. 280 805 1969

Antanovski, A Social class and the major cardiovascular diseases. J chron. Dis. 21 65 1968.

Armitage, P Statistical methods in medical research. Blackwell, Oxford, 1971.

Armstrong, A. Edinburgh community study of acute coronary disease. In Acute myocardial infarction, proceedings of symposium. Sept. 1967 University of Edinburgh. Ed. D. G Julian and M. F Oliver Livingstone, Edinburgh 1968.

Armstrong, A Duncan, B Oliver M. F Julian, D G Donald, K. W Fulton, M., Lutz, W & Morrison, S. L. N tural history of acute coronary heart attacks. A community study Brit. Heart J 34 67 1972.

Arterial hypertension and ischaemic heart disease. Preventive aspects. Report of an expert committee. Wld Hlth Org. techn. Rep. Ser 231 1 1962.

Auerbach, O Hammond, E. C. & Garfinkel L. Smoking in relation to therosclerosis of the coronary arteries. New Engl. J Med. 273 775, 1965.

Auerbach, O Hammond, E. C. & Garfinkel, L. Thickening of walls f arterioles and small arteries in relation to age and smoking habits. New Engl. J Med. 278 980, 1968.

Badger G. F & Liebow I. M. Myocardial infarction in the practices of a group of private physicians. 4. J chron. Dis. 21 473, 1968.

Bainton, C. R. & Peterson, D R. Deaths from coronary heart disease in persons fifty years of age and younger A community wide study New Engl. J Med. 268 569 1963

Baroldi, G Acute coronary occlusion as a cause of myocardial infarct and sudden coronary heart death. Amer J Cardiol. 16 859 1965.

Baroldi, G The coronary arterial collateral circulation in man. Acta cardiol. (Brux) Suppl. 13 17 1969.

Bondurant, S. Problems of the pre-hospital phase of acute myocardial infarction. Amer J Cardiol. 24 612, 1969.

Bradley R. F & Partamian, J O. Coronary heart disease in the diabetic patient. Med. Clin. N Amer 49 1093, 1965.

Bradley R. F & Schonfeld, A.. Diminished pain in diabetic patients with acute myocardial infarction. Geriatrics 17 322, 1962.

Brown, D F Kinch, S. H. & Doyle, J T.. Serum triglycerides in health and in ischemic heart disease. New Engl. J Med. 273 947 1965.

Brown, R. G., Davidson, L. A. G McKeown, T & Whitfield, A. G W.. Coronary-artery disease influences affecting its incidence in males in the seventh decade. Lancet 2. 1073, 1957

Brunner D. & Manelis, G. Myocardial infarction among members of communal settlements in Israel. Lancet 2 1049 1960.

Brylogle, J W & Bradley R F The vascular complications of diabetes mellitus a clinical study Diabetes 6 159 1957

Carroll, S. E., Ahuja, S. P & Manning, G W The initiation of ventricular tachycardia and fibrillation in experimental coronary artery occlusion. Amer J Cardiol. 16 813, 1965.

Chapman, J M., Goerke, L. S., Dixon, W Loveland, D B. & Phillips, E. The clinical status of a population group in Los Angeles under observation for two to three years. Amer J publ. Hlth 47 Suppl. April, 33, 1957

Chiang, B N Perlman, L. V Fulton, M., Ostrander L. D & Epstein, F H. Predisposing factors in sudden cardiac death in Tecumseh, Michigan. Circulation 41 31 1970

Chiang, B. N Perlman, L. V Ostrander L. D Jr & Epstein, F H. Relationship of premature systoles to coronary heart disease and sudden death in the Tecumseh epidemiologic study Ann. intern. Med. 70 1159 1969.

Cooley W W & Lohnes, P R. Multivariate data analyses. Wiley New York 1971.

Crawford, T Dexter D & Tearre, R. D Coronary artery pathology in sudden death from myocardial ischaemia. A comparison by age-groups. Lancet 1 181 1961

Croce, L., Noseda, V Bertelli, A. & Bossi, E. Sudden and unexpected death from heart disease. Epidemiological, anatomo- and physiopathologic aspects A study of 1047 cases. Cardiologia (Basel) 37 331 1960

Cross, E. B Coronary care units The U.S. Public Health Services Co-operative study In Acute myocardial infarction proceedings of a symposium. Sept 1967 University of Edinburgh. Ed D G Julian and M F Oliver Livingstone, Edinburgh 1968.

Dawber T R. & Thomas, H. E Jr Prevention of myocardial infarction Progr cardiovasc. Dis. 13 343, 1971

Deming, Q B Blood pressure Its relation to atherosclerotic disease of the coronaries. Bull. N Y Acad Med 44 968 1968

Dixon, T C Cardiac arrest Experience and results in emergency treatment outside the operating theatre Med J Aust 1 754, 1970

Doyle, J T Smoking and myocardial infarction. Circulation 39-40 Suppl 4 136 1969

Doyle, J T Dawber T R Kannel, W B Heslin, A. S & Kahn H A Cigarette smoking and coronary heart disease Combined experience of the Albany and Framingham studies. New Engl. J Med 66 ?86 196?

Doyle J T Dawber T R Kannel, W B Kinch, S. H. & Kahn, H A The relationship of cigarette smoking to coronary heart disease. The second report of the combined experience of the Albany NY and Framingham, Mass., studies. J Amer med. Ass. 190 886, 1964.

Doyle, J T Heslin, A. S., Hilleboe, H. E. & Formel, P F Early diagnosis of ischemic heart disease. New Engl. J Med. 261 1096, 1959

Eckstein, R. W Effect of exercise and coronary artery narrowing on collateral circulation. Circulat. Res. 5 230 1957

Edwards, J E. The value and limitations of necropsy studies in coronary arterial disease. Progr cardiovasc. Dis. 13 309 1971

Eisenberg, H., Feltner W R., Payne G H., Haddad, C. A. & Conn, H. The epidemiology of coronary heart disease in Middlesex County Connecticut. J chron. Dis. 14 221 1961.

Epstein, F H. Hereditary aspects of coronary heart disease. Amer Heart J 67 445, 1964.

Epstein, F H. The epidemiology of coronary heart disease. A review J chron. Dis 18 735 1965

Epstein, F H. Multiple risk factors and the prediction of coronary heart disease. Bull. N.Y Acad. Med. 44 916, 1968.

Epstein, F H. Elevated blood sugar The associated cardiovascular risk. Minn. Med. 52 1271 1969

Epstein, F H. & Ostrander L. D Jr Detection of individual susceptibility toward coronary disease. Progr cardiovasc. Dis. 13 324, 1971.

Feil, H. Preliminary pain in coronary thrombosis. Amer J med. Sci. 193 42, 1937

Fodor J The ischaemic heart disease register in Göteborg. A pilot study 1 11. -68—31. 1 -69 Pehr Dubb Journalen 3 26, 1969

Franco, A. La mort subite due a'une maladi du coeur Arch. Mal. Coeur 55 632, 1962.

Frank, C. W The course of coronary heart disease factors relating to prognosis. Bull. N Y Acad. Med. 44 900 1968.

Frank, C W Weinblatt, E., Shapiro, S. & Sager R. V Physical inactivity as a lethal factor in myocardial infarction among men. Circulation 34 1022, 1966.

Friedman, M. Behavior pattern and its pathogenetic role in clinical coronary artery disease. Geriatrics 19 562, 1964.

Friedman, M. & Rosenman, R H. Overt behavior pattern in coronary disease Detection of overt behavior pattern A in patients with coronary disease by new psyhophysiological procedure. J Amer med. Ass. 173 1320 1960.

Fry J Acute myocardial infarction in a British general practice. Experience during 20 years. In Acute myocardial infarction proceedings of symposium. Sept 1967 University of

Edinburgh. Ed. D. G Julian and M. F Oliver Livingstone, Edinburgh 1968.

Fry J & Dillane J B. Acute coronary deaths. Possible salvage? J roy Coll. gen. Practit. 14 44, 1967

Fulton, M Duncan, B., Lutz, W Morrison, S. L., Donald, K. W Kerr F Kirby B. J Julian, D. G & Oliver M. F Natural history of unstable angina. Lancet 1 860, 1972.

Fulton, M., Julian, D. G. & Oliver M. F Sudden death and myocardial infarction. Circulation 49/50 Suppl. 4 182, 1969

Gorbatow O Die Herzinfarktfrequenz in einem Material der Bevolkerung von Helsinki während der Jahre 1945—1952. Acta med. scand. Suppl. 364 169 1961.

Gorbatow O., Henrieson, T & Abo, S. L. Hjärt infarct i Esbo år 1965. Epidemiologiska synpunkter Finska läkaresällsk. Handl. 113 43, 1969

Gorbatow O Henrieson, T Abo, S. L., Kause, P & Laulajainen, M. Coronary heart disease in Helsinki. A comparative investigation of the years 1935 and 1965. Acta Soc. Med. scand. 1 27 1971.

Gordon, T & Kannel, W B. Premature mortality from coronary heart disease. J Amer med. Ass. 215 1617 1971.

Hackett, T P & Cassem, N H. Factors contributing to delay in responding to the signs and symptoms of acute myocardial infarction. Amer J Cardiol. 24 651, 1969

Hagstrom, R. M., Billings, F T J Ball, C. O T & Meneely G R. The risk of sudden death following myocardial infarction. Arch. environm. Hlth 15 450, 1967

Hallermann, W Über die typische Lokalisation der Coronartrombose bei tödlichem Herzinfarkt. Dtsch Z. ges. gerichtl. Med. 52 393, 1962.

Han, J Mechanisms of ventricular arrhytmias associated with myocardial infarction. Amer J Cardiol. 24 800, 1969.

Hansen, J P H. Coronary death in younger persons. Dan. med Bull. 15 201, 1968.

Hellerstein, H. K. & Turell, D J.. The mode of death in coronary artery disease In Sudden cardiac death. Ed. B. Surawicz and E. D. Pellegrino. Grune, New York 1964.

Herrick, J B.. Clinical features f sudden obstruction of the coronary arteries. J Amer med. Ass. 59 2015, 1912.

Hinkle L. E J Whitney L. H., Lehman, E. W Dunn, J King, B. B. R., Plakun, A. & Flehinger B Occupation, education, and coronary heart disease. Science 161 238, 1968.

Hochberg, H M. Characteristics and significance of prodromes of coronary care unit patients. Chest 59 10, 1971

Hypertension and coronary heart disease classification and criteria for epidemiological studies. Wld Hlth Org. techn. Rep. Ser 168, 1959

Ischaemic heart disease A secondary prevention trial using clofibrate. Report by a research committee of the Scottish Society of Physicians. Brit. med. J 4 775 1971

James, T N Pathology of small coronary arteries. Amer J Cardiol. 20 679 1967

James, T N The coronary circulation and conduction system in acute myocardial infarction. Progr cardiovasc. Dis. 10 410 1968.

James, T N Pathogenesis of arrhytmias in acute myocardial infarction. Amer J Cardiol. 24 791, 1969

James, T N Sudden death related to myocardial infarction. Circulation 45 205, 1972.

Kagan, A., Livsic, A. M., Sternby N & Vihert, A. M Coronary-artery thrombosis and the acute ttack of coronary heart-disease. Lancet 2 1199, 1968.

Kaltaranta, H., Purola, T Kalimo, E. & Kauppinen, H. Smoking patterns among adult urban population in Finland. Scand. J clin. Lab. Invest. 29 Suppl. 122 23 1972.

Kannel, W B Habitual level of physical activity and risk of coronary heart disease the Framingham study Canad. med. Ass. J 96 811 1967

Kannel, W B. Physical exercise and lethal therosclerotic disease. New Engl. J Med. 282 1153, 1970

Kannel, W B Castelli, W P & McNamara, P M. The coronary profile 12 year follow-up in the Framingham study J occup. Med. 9 611 1967 a.

Kannel, W B., Castelli, W P & McNamara, P M. Serum lipid fractions and risk of coronary heart disease. The Framingham study Minn. Med. 52 1225 1969

Kannel, W B Dawber T R. & McNamara, P M. Detection of the coronary-prone adult The Framingham study J Iowa St. med. Soc. 56 26 1966.

Kannel, W B. & Feinleib, M. N tural history of angina pectoris Prognosis and survival. The Framingham study Amer. J Cardiol. 26 640, 1970.

Kannel, W B. & Feinleib, M.. Natural history of angina pectoris in the Framingham study Prognosis and survival. Amer. J Cardiol. 29 154, 1972.

Kannel, W B., Gordon, T & Schwartz, M. J Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study Amer J Cardiol. 27 335, 1971.

Kannel, W B LeBauer E. J Dawber T R. & McNamara, P M. Relation of body weight to development of coronary heart disease. Circulation 35 734, 1967 b.

Kannel, W B., McNamara, P M., Feinleib M. & Dawber T R. The unrecognized myocardial infarction. Fourteen year follow up experience in the Framingham study Geriatrics 25 75 1970.

Karvonen, M. J Orma, E., Punsar S., Kallio V Arstila, M., Luomanmäki, K. & Takkunen, J Coronary heart disease in seven countries. 6. Five-year experience in Finland Circulation 41 Suppl. 1 52, 1970

Keith, R. A. Personality and coronary heart disease A review J chron. Dis. 19 1231 1966.

Keys, A. Obesity and degenerative heart disease. Amer J publ. Hlth 44 864 1954.

Keys, A. (Ed) Coronary heart disease in seven countries. Circulation 41 Suppl. 1 1970

Keys, A Fidanza, F Karvonen, M. J Kimura, N & Tayl H. L. Indices f relative weight and obesity J Chron. Dis. 25 329 1972.

Kinlen, L. J On community study of myocardial infarction & sudden death. MS.D Phil., thesis 1969 University of Oxford, 1969

Kjeldsen, K. Smoking and theroscleroeis Investigations of the significance of the carbon monoxid content in tobacco smoke in therogenesis. Munksgaard, Copenhagen 1969

Kraus, A. S & Lilienfeld, A. M. Some epidemiologic aspects of the high mortality rate in the young widowed group J chron. Dis 10 207 1959

Kuller L. Sudden and unexpected non-traumatic deaths in adults A review f epidemi logical and clinical studies J chron. Dis. 19 1165 1966.

Kuller L. Sudden death in arteriosclerotı heart disease. Amer J Cardiol. 24 617 1969

Kuller L Lilienfeld, A & Fisher R. Epidemilogical study of sudden and unexpected deaths due to arteriosclerotic heart disease Circulation 34 1056 1966

Kuller L., Lilienfeld, A & Fisher R Sudden and unexpected deaths due to natural causes in adults. A comparison of deaths certified and not certified by the medical examiner Arch. environm. Hlth 13 236, 1966 b.

Kuller L., Lilienfeld, A. & Fisher R Sudden and unexpected deaths in young adults. J Amer med. Ass. 198 248, 1966

Kuller L., Lilienfeld, A. & Fisher R. An epidemiological study of sudden and unexpected deaths in dults. Medicine (Baltimore) 46 341 1967

Lawrie, D M., Higgins, M. R., Godman, M. J Oliver M. F Julian, D G & Donald, K. W Ventricular fibrillation complicating acute myocardial infarction Lancet 2 523, 1968.

Levine, H. J Pre-hospital management of acute myocardial infarction. Amer J Cardiol. 24 826, 1969

Liebow I. & Badger G F Myocardial infarction in the practices of a group of private physicians. A comparison of patients with and without diabetes. 1 The first sixty days. J chron. Dis. 16 1013, 1963.

Lindholm, B. The ischaemic heart disease register How do patients with myocardial infarction come to hospital care? Pehr Dubb Journalen 3 34, 1969

Lindström, B Death from ASHD outside hospital. Pehr Dubb Journalen 3 38 1969

Lovell, R. R. H. & Prineas, R. J Mechanisms of sudden death and their implications for prevention and management Progr cardiovasc. Dis. 13 482, 1971.

Lown, B. The philosophy of coronary care Arch. klin. Med. 216 201, 1969

Lown, B Fakhro, A. M Hood, W B., Jr & Thorn, G W Th coronary care unit. New perspectives and directions. J Amer med. Ass 199 188, 1967

Lown, B., Klein, M. D & Hershberg, P L Coronary and precoronary care. Amer J Med. 46 705, 1969 a.

Lown, B., Kosowsky B. D. & Klein, M. D. Pathogenesis, prevention, and treatment of arrhythmias in myocardial infarction. Circulation 39/40 Suppl. 4 261 1969 b.

Lown, B & Wolf, M. Approaches to sudden death from coronary heart disease. Circulation 44 130 1971.

Luke, J L. & Helpern, M. Sudden unexpected death from natural causes in young adults. A review of 275 consecutive autopsied cases. Arch. Path 85 10 1968.

Lumb, G & Shacklett, R. S The relationship of sudden death to lesions of the cardiac conduction tissue. N C. med. J 23 69, 1962.

McDonough, J R., Hames, C. G Stulb, S. C. & Garrison, G E. Coronary heart disease among negroes and whites in Evans County Georgia. J chron. Dis. 18 443, 1965

McNeilly R. H. A study of the epidemiology f 998 fatal cases of coronary artery disease in Belfast and the relationship of some biological and social factors to the duration of the

last ttack. M. D Thesis, Queen s University Belfast 1967
McNeilly R. H. & Pemberton, J Duration of last ttack in 998 fatal cases of coronary artery disease and its relation to possible cardiac resuscitation. Brit. med. J 3 139 1968
McWhinney I. R. Incidence of ischaemic heart disease in a country town group practice. Lancet 2 342, 1968.
Marks, H. H. Influence of obesity on morbidity and mortality Bull. N Y Acad Med. 36 296 1960
Marks, R U Social stress and cardiovascular disease. A review of empirical findings. Mil bank mem. Fd. Quart. 45 Suppl. 51 1967
Master A. M. & Geller A. J The extent of completly asymptomati coronary artery disease. (Editorial) Amer J Cardiol. 23 173, 1969
Master A. M. & Jaffe H. L. Physiologic effects of obesity upon heart, incidence of obesity in coronary disease. J Amer Geriat. Soc. 3 299 1955.
Master A. M Jaffe, H. L. & Chesky K. Relationship of obesity to coronary disease and hypertension. J Amer med. Ass 153 1499 1953.
Mathewson, F A. L., Brereton, C. C., Keltie W A. & Paul, G I. The university of Manitoba follow-up study A prospective investig tion of cardiovascular disease. Part I. General description — mortality and incidence f coronary heart disease. Canad med. Ass. J 92 947 1965.
Meadows, R. Coronary thrombosis and myocardial infarction. Med. J Aust. 2 409 1965.
Mitchell, J R. A. & Schwartz, C. J Arterial disease. Blackwell, Oxford, 1965.
Montenegro, M. R & Solberg, L. A Obesity body weight, body length, and atherosclerosis. Lab. Invest. 18 594, 1968.
Morris, J N & Crawford, M. D Coronary heart disease and physical activity of work. Brit. med. J 2 1485 1958.
Morris, J N & Gardner M. J Epidemiology of ischaemic heart disease. Amer J Med. 46 674, 1969
Morris, J N Heady J A Raffle, P A. B., Roberts, C. G & Parks, J W Coronary heart disease and physical activity of work. Lancet 2 1111, 1953.
Mosbech, J & Dreyer K. Akut koronarokklusion in Danmark. Morbiditet og mortalitet. 2. Ugeskr Laeg. 127 1473, 1965.
Moss, A. J Wynar B. & Goldstein, S Delay in hospitalization during the acute coronary period. Amer J Cardiol. 24 658, 1969
Mulcahy R & Hickey N Cigarett smoking habits f patients with coronary heart disease. Brit. H art J 28 404 1966.
Myerburg, R. J & Davis, J H. The medical ecology of public safety I. Sudden death due to coronary heart disease. Amer Heart J 68 586, 1964.
Oberman, A., Harlan, W R., Smith, M. & Graybiel, A. The cardiovascular risk associated with different levels and types of elevated blood pressure. Minn. Med. 52 1283 1969
Olin, H. S. & Hackett, T P The denial f chest pain in 32 patients with acute myocardial infarction. J Amer med. Ass. 190 977 1964
Oliver M. F The place of the coronary care unit. J roy Coll. Phycns Lond. 3 47 1968
Oram, S. Smoking and ischaemic heart disease. Brit. Heart J 30 145 1968.
Oscherwitz, M Kramoff, S. O Moretti, L & Syme, S. L The relationship of myocardial infarction to parental mortality and longevity J chron. Dis. 21 341 1968.
Ostfeld, A. M Lebovits, B. Z., Shekelle, R. B & Paul, O. A prospective study f the relationship between personality and coronary heart disease. J chron Dis. 17 265, 1964
Ostrander L. D J Francis, T Jr Hayner N S Kjelsberg, M. O & Epstein, F H The relationship of cardiovascular disease to hyperglycemia. Ann. intern. Med. 62 1188, 1965
Oxman, H. A., Titus, J L., Nobrega, F T & Connolly D C. Prognostic effect of electrocardiographic bnormalities at the time of initial diagnosis of angina pectoris. Amer J Cardiol. 26 653 1970
Pantridge, J F & Adgey A. A. J Pre-hospital coronary care. The mobile coronary care unit. Amer J Cardiol. 24 666, 1969
Pantridge, J F & Geddes, J S. A mobil intensive care unit in the management of myocardial infarction. Lancet 2 271 1967
Parkes, C. M., Benjamin, B & Fitzgerald, R. G Broken heart A statistical study of increased mortality among Widowers. Brit. med. J 1 740, 1969
Pathological diagnosis of acute ischaemic heart disease Report of a WHO Scientific Group. Wld Hlth Org. techn. Rep. Ser 441, 1970.
Paul, O Physical inactivity the associated cardiovascular risk. Minn. Med. 52 1327 1969
Paul O., Lepper M. H., Phelan, W H Dupertuis, G W MacMillan, A., McKean, H. & Park, H. A longitudinal study of coronary heart disease. Circulation 28 20, 1963.
Paul O. & Schatz, M.: On sudden death. Circulation 43 7 1971

Pell, S. & D'Alonzo, C. A. Immediate mortality and five-year survival of employed men with a first myocardial infarction. New Engl. J Med. 270 915 1964.

Peterson, D. R., Thompson, D. J & Nam, J M. Water hardness, arteriosclerotic heart disease and sudden death. Amer J Epidem. 92 90, 1970.

Punsar S. Personal communication. 1971

Punsar S Prognosis of CHD in East and West Finland. Scand. J clin. Lab Invest. 29 Suppl. 122 19 1972.

Punsar S. & Pyörälä, K. Cigarette smoking and the risk of death from coronary heart disease in East and West Finland. Scand. J clin. Lab Invest. 25 Suppl. 113 38, 1970

Rabson, S. M. & Helpern, M. Sudden and un expected natural death. 2. Coronary artery sclerosis. Amer Heart J 35 635, 1948.

Rahe, R. H. & Lind, E. Psychosocial factors and sudden cardiac death A pilot study J psychosom. Res. 15 19 1971.

Rahe, R. H., Romo, M., Bennett, L. & Siltanen, P Subjects recent life changes and coronary death in Helsinki. (In preparation).

Rees, W D. & Lutkins, S. G Mortality of bereavement. Brit. J Med. 4 13 1967

Rissanen, V T Occurrence of coronary occlusion in cases of coronary death and accidental death. Adv Cardiol. 4 99, 1970. Thrombosis and Coronary Heart Disease. First Paavo Nurmi symposium, Porvoo, Finland 1969 (Karger Basel 1970)

Rissanen, V Pyörälä, K. & Heinonen, O P Cigarette smoking in relation to coronary and aortic atherosclerosis. Acta path. microbiol. scand. Sect. A 80 491 1972.

Rissanen, V T Romo, M Siltanen, P Pyörälä, K. & Halonen, P L Sudden coronary death on the basis f WHO Ischaemic Heart Disease Register in Helsinki Scand. J clin. Lab. Invest. 27 Suppl. 116 18, 1971.

Roberts, W C Coronary arteries in fatal acute myocardial infarction. Circulation 45 215, 1972.

Robertson, W B & Strong, J P Atherosclerosis in persons with hypertension and diabetes mellitus. Lab. Invest. 18 538, 1968

Romo, M., Siltanen, P Pyorala, K & Halonen, P L Helsingin koronaarirekisteri Esitutkimus 1 9.—31. 12. 1969 (Register of Coronary Cases in Helsinki.) Suom. Lääk. L. 25 2520, 1970.

Romo, M., Siltanen, P Rissanen, V Pyorala, K & Halonen, P L The mortality from coronary disease in Helsinki on the basis of WHO Ischaemic Heart Disease Register Scand. J clin. Lab. Invest. 27 Suppl. 116 18, 1971

Rose, G Familial patterns in ischaemic heart disease. Brit. J prev Soc. Med. 18 75, 1964.

Rosenman, R. H., Friedman, M., Jenkins, C. D Straus, R., Wurm, M. & Kositchek, R. Clinically unrecognized myocardial infarction in the Western Collaborative group study Amer J Cardiol. 19 776, 1967 a.

Rosenman, R. H., Friedman, M., Jenkins, C. D Straus, R Wurm, M. & Kositchek, R. Recurring and fatal myocardial infarction in the Western Collaborative group study Amer J Cardiol. 19 771 1967 b.

Rosenman, R. H., Friedman, M. Straus, R., Wurm, M Kositchek, R., Hahn, W & Werthessen, N T A predictive study of coronary heart disease — the Western Collaborative group study J Amer med. Ass. 189 15 1964.

Sackett, D L., Gibson, R. W Bross, I. D. J & Pickren, J W Relation between aortic athero-sclerosis and the use of cigarettes and alcohol. An autopsy study New Engl. J Med. 279 1413 1968.

Sampson, J J & Eliaser M., Jr The diagnosis of impending acute coronary artery occlusion. Amer Heart J 13 675 1937

Sanders, K. Coronary-artery disease and obesity Lancet 2 432, 1959.

Sarna, S. Erotteluanalyysi ja luokittelu. Research report. Applied Mathematics Department, Uni versity of Helsinki 1970.

Schwartz, C. J & Walsh, W J The pathologic basis of sudden death. Progr Cardiovasc. Dis. 13 465 1971

Scott, R. F & Briggs, T S. Pathologic findings in pre-hospital deaths due to coronary thero-sclerosis. Amer J Cardiol. 29 782, 1972.

Seim, S. Angina pectoris. A prognostic study Acta med. scand. 168 255, 1960

Seltzer C. C. An evaluation of the effect of smoking on coronary heart disease. 1 Epidemi ological evidence. J Amer med. Ass. 203 193, 1968.

Seltzer C. C. Overweight and obesity The associated cardiovascular risk. Minn. Med. 52. 1265 1969

Seltzer C. C. The effect of cigarette smoking on coronary heart disease. Where do we stand now? Arch. environm. Hlth 20 418, 1970.

Shapiro, S., Weinblatt, E., Frank, C. W & Sager R. V The H.I.P study of incidence and prognosis of coronary heart disease. Prelimi nary findings on incidence of myocardial in farction and angina. J chron. Dis. 18 527 1965.

Shapiro, S., Weinblatt, E., Frank, C. W & Sager R. V Incidence of coronary heart disease in population insured for medical care (HIP)

myocardial infarction, angina pectoris, and possible myocardial infarction. Amer J publ. Hlth 59 Suppl. 2 1, 1969.

Sheps, M. C. Marriage and mortality Amer J publ. Hlth 51 547 1961.

Sidel, V W Acton, J & Lown, B. Models for the evaluation of pre-hospital coronary care. Amer J Cardiol. 24 674, 1969.

Sievers, J.. Myocardial infarction. Clinical features and outcome in three thousand thirty-six cases. Acta med. scand. 175 Suppl. 406, 1963.

Siltanen, P The ischaemi heart disease register as frame for preventive measures. Ad Car diol. 8 214, 1972. Early diagnosis of coronary heart disease. Karger Basel (In press.).

Siltanen, P Romo, M., Pyörälä, K. & Halonen, P L. A pre-pilot tudy on ischemic heart disease register in Helsinki. Scand. J clin. Lab. Invest. 25 Suppl. 113 38 1970.

Simborg, D W The status of risk factors and coronary heart disease. J chron. Dis. 22 515, 1970.

Sipilä, W Sydäninfarktin ennuste. (Prognosis f Myocardial Infarction.) Diss., Helsinki 1966.

Slack, J & Evans, K. A. The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease. J med. Genet. 3 239 1966.

Solomon, H. A Edwards, A. L. & Killip, T Prodromata in acute myocardial infarction. Circulation 40 463, 1969

Spain, D. M.. Anatomical basis of sudden cardiac death. In. Sudden cardiac death. Ed. B. Surawicz and E. D Pellegrino. Grune, New York 1964.

Spain, D M. & Bradess, V A. Sudden death from coronary therosclerosis. Age, race, sex, physical activity and alcohol. Arch. intern. Med. 100 228, 1957

Spain, D M. & Bradess, V A. Frequency of coronary thrombi as related to duration of survival from onset f acute fatal episodes f myocardial ischemia Circulation 22 816, 1960.

Spain, D. M. & Bradess, V A Sudden death from coronary heart disease. Survival time, frequency f thrombi, and cigarette smoking. Chest 58 107 1970

Spain, D M., Bradess, V A., Iral, P & Cruz, A.. Intercoronary anastomotic channels and sudden unexpected death from advanced coronary therosclerosis. Circulation 27 12, 1963.

Spain, D M., Bradess, V A., Matero, A. & Tarter R Sudden death due to coronary atherosclerotic heart disease. Age, smoking habits, and recent thrombi. J Amer med. Ass. 207 1347 1969

Spain, D M Bradess, V A. & Mohr C. Coronary therosclerosis as a cause of unexpected and unexplained death. An utopsy study from 1949—1959 J Amer med. Ass. 174 384, 1960.

Spiekerman, R. E., Brandenburg, J T Achor R. W P & Edwards, J E. The spectrum of coronary heart disease in a community of 30 000. A clinicopathological study Circulation 25 57 1962.

Stamler J Lectures on preventive cardiology Grune, New York 1967

Stamler J.. Cigarette smoking and therosclerotic coronary heart disease. Bull. N Y Acad. Med. 44 1476, 1968.

Stamler J.. Acute myocardial infarction — progress in primary prevention. Brit. Heart J 33 Suppl. 145 1971

Stamler J Lindberg, H. A., Berkson, D. M., Shaffer A., Miller W & Poindexter A. Prevalence and incidence of coronary heart disease in strata of the labor force of Chicago industrial corporation. J chron. Dis. 11 405, 1960.

Stamler J Schoenberger J A., Lindberg, H. A., Shekelle, R., Stoker J M., Epstein, M. B., d Boer L., Stamler R., Restivo, R., Gray D. & Cain, W Detection of susceptibility to coronary disease. Bull. N Y Acad. Med. 45 1306 1969

Stowers, M. & Short, D Warning symptoms before majo myocardial infarction. Brit. Heart J 32 833, 1970.

Strong, J P McGill, H. C., Jr Richards, M. L. & Eggen, D. A. Relationship between cigarette smoking habits and coronary atherosclerosis in utopsied males (P). Circulation 33 Suppl. 3 31 1966.

Surawicz, B. & Pellegrino, E. D (Ed) Sudden cardiac death. Grune, New York 1964.

Taylor H. L. Occupational factors in the study f coronary heart disease and physical activity Canad. med. Ass. J 96 825 1967

Tenhu, M Kataja, M., Lehti, H., Rissanen, V & Pyörälä, K.. The medico-legally autopsied coronary deaths in Helsinki, 1959—1968. Scand. J clin. Lab. Invest. 25 Suppl. 113 39 1970 a.

Tenhu, M., Rissanen, V & Pyörälä, K. The coronary deaths in Helsinki on the basis of death certificates in 1959—1967 Scand. J clin. Lab. Invest. 25 Suppl. 113 39 1970 b.

Tibblin, G Risk factors in coronary heart disease. Ad Cardiol. 4 123, 1970. Thrombosis and Coronary Heart Disease. First Paavo Nurmi symposium, Porvoo, Finland 1969 (Karger Basel 1970)

Titus, J L., Oxman, H. A., Nobrega, F T & Connolly D C. Sudden unexpected deaths as the initial manifestation of ischemic heart disease. Clinical and pathologic observations. Amer J Cardiol. 26 662, 1970.

Trial of clofibrate in the treatment of ischaemic heart disease. Five-year study by a group of physicians of the Newcastle upon Tyne Region. Brit. med. J 4 67 1971.

U.S. Public Health Service. Surgeon General's Advisory Committee on Smoking and Health. Smoking and health report. Public Health Service publication, No. 1103 U.S Govt. Print. Off., Washington 1964.

U.S. Public Health Service. The health consequences of smoking a Public Health Service review 1967 Public Health publication, No. 1696 U S. Govt. Print. Off., Washington 1967

U.S. Public Health Service. The health consequences of smoking Public Health Service review 1967 Public Health publication, No 1696-2. Suppl. 1969 U.S Govt. Print. Off., Washington 1969

Uotila, U Über den plötzlichen unerwarteten Tod aus natürlicher Ursache. Acta Soc. Med. Duodecim. Ser B 33 1 1945.

Uotila, U Äkilliseeta, odottamattomasta kuolemasta. In Oikeuslääketiede. Ed U Uotila and M. Kakomaki. 2. ed. WSOY Porvoo 1970.

Varpela, E Hjart och kärlsjukdomsdödligheten i Helsingfors Nord. Med. 63 127 1960.

Vasama, P & Vartia, Y Johdatus tilastotieteeseen. 2. Yliopplastuki ry Helsinki 1972.

Webb W R Wax, S. D & Sugg W L. Cigarette smoke and fibrillation threshold in dogs. Clin. Res 16 74 1968

Weinberg, S B. & Helpern, M. Circumstances related to sudden, unexpected death in coronary heart disease. In Work and the heart Transactions f the first Wisconsin conference on work and the heart. Ed F F Rosenbaum and E L Belknap Hoeber New York 1959

Weinblatt, E Shapiro, S Frank, C W & Sager R V Prognosis of men after first myocardial infarction Mortality and first recurrence in relation to selected parameters Amer J publ Hlth 58 1329 1968

Weiss M M Ten-year prognosis of acute myocardial infarction Amer J med. Sci 231 9 1956

Wikland, B Death from arteriosclerotic heart disease outside hospitals A study f 2678 cases in Stockholm with particular reference to sudden deaths Acta med. scand. 184 129 1968.

Wikland, B. Medically unattended fatal cases of ischaemic heart disease in a defined population. Acta med. scand. Suppl. 524, 1971

Wolf, S. Psychosocial forces in myocardial infarction and sudden death. Circulation 39/40 Suppl. 4 74, 1969

Working Group of Ischaemic Heart Disease Registers. 27—31 May 1968 Copenhagen. Report of a working group Regional Office for Europe World Health Organization. EURO 5010 (1) Copenhagen 1968

Working Group on Ischaemic Heart Disease Registers. 12—14 May 1969 Copenhagen. Report on a working group Parts I and II. Regional Office for Europe World Health Organization. EURO 5010 (2), Copenhagen 1969

Working Group of Ischaemic Heart Disease Registers. 29 June — 1 July 1970, Copenhagen. Report of the 4th working group with revised operating protocol. Regional Office for Europe, World Health Org nization. EURO 5010 (4) Copenhagen 1970

Working Group on Ischaemic Heart Disease Registers. 1971 Copenhagen. Report of the 5th working group with second revision of the operating protocol. Regional Office for Europe, World Health Organization. EURO 5010 (5), Copenhagen 1971

Yater W M., Traum, A. H Brown, W G Fitzgerald, R. P Geisler M. A & Wilcox, B. B. Coronary artery disease in men eighteen to thirty nine years of age. Report of eight hundred sixty-six cases, four hundred fifty with necropsy examinations. Amer Heart J 36 334, 1948.

Yu, P N Prehospital care of acute myocardial infarction. Circulation 45 189 1972.

Zalokar J B. Marital status and major causes of death in women. J chron. Dis. 11 50 1960

Zukel, W J Cohen, B. M., Mattingly T W & Hrubec, Z. Survival following first diagnosis of coronary heart disease. Amer Heart J 78 159 1969

Zukel, W J Lewis, R. H., Enterline P E., Painter R. C., Ralston, L. S., Fawcett, R. M., Meredith, A P & Peterson, B. A short-term community study of the epidemiology of coronary heart disease. Amer J publ. Hlth 49 1630 1959

Acta Medica Scandinavica

Supplementum 548

Haemodynamic Effects of Physical Training After Myocardial Infarction

By Arne Bjernulf

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica.*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each annually) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw cr 170 incl. postage.
Other countries Sw cr 200 incl. postage.

Chief Editor

Professor Jan G Waldenström, MD
Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Kungsholmsstrand 175
S-112 48 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell Informationsindustri AB
S-751 81 Uppsala, Sweden

FROM THE DEP RTMENTS OF INTERNAL MEDICINE AND CLINICAL PHYSIOLOGY
UNIVERSITY HOSPITAL UPPSALA SWEDEN

Haemodynamic aspects of physical training after myocardial infarction

By Arne Bjernulf

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each annually) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw cr 170, incl. postage.
Other countries: Sw cr 200, incl. postage.

Chief Editor

Professor Jan G Waldenström, MD
Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Kungsholmsstrand 175
S-112 48 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26 Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell Informationsindustri AB
S-751 81 Uppsala, Sweden

CONTENTS

ABSTRACT

The study was made on 63 male patients treated at the University Hospital, Uppsala, for acute myocardial infarction, with an onset during the period 15/3 1969–15/3 1972.

Patients with the following were excluded: previous cardiac infarction; diastolic blood pressure at rest of more than 110 mm Hg; a relative heart volume of more than 600 ml; manifest diabetes mellitus, other complicating diseases, e.g. pulmonary disease or arthrosis of the hips or back. All patients equal to or more than 70 years of age were also excluded.

The 63 patients selected were admitted to hospital 3 months after the onset of the infarction for examination including right heart catheterization. They were then classified randomly to trained (34 patients) or eference group (29 patients).

Starting 3 months after the onset of the infarction, the patients of the trained group underwent a 3-month period of individual physical training (3 one-hour sessions per week under the leadership of a physiotherapist). The patients of the reference group carried out only their daily physical activity during this period (the reference period). Six months after the onset of the infarction the patients of both groups were readmitted to hospital for second examination including heart catheterization.

During the approximately 1000 training sessions no arrhythmias or other serious complications occurred. The emergency equipment that was always available (including defibrillator) was never needed.

The haemodynamic investigation showed that during work the pressure in th pulmonary circulation, including the PCV pressure, was pathologically increased. No change after the training period was noted. In both the trained and referenc group lower heart rat at rest was recorded after the training or reference period. During work a decrease was noted in both groups, but this was more marked in the trained group. The strok vol me during submaximal work increased in th trained group, especially in the younger patients without angina pectoris. Th cardiac output at rest and during work remained unchanged in both the trained and reference group. Neither was there any alteration of the arteriovenous oxygen difference. Th left ventricular minute work was decreased after the training due to reduction of the arterial blood pressure during exercise. The reference group showed no significant reductions of left entricular minut work or blood pressure. Finally the peripheral and central circulatory effects of th training, and their possible nderlying mechanisms, were discussed.

INTRODUCTION

Physical training in ischaemic heart disease is a form of treatment that has become increasingly common During the last decade several authors have reported an increased physical work capacity decreased severity of angina pectoris and an increased feeling of general well-being after a period of physi cal training (1 13 14 16 26 35 36, 45 54 55 62, 68 70) An important element of the beneficial effect of physical training — which is usually carried out in a group under the close supervision of a doctor — would seem to be psychological, resulting partly from a reduction of the anxiety and apprehension associated with physical effort. The beneficial effect probably also has an element with an objective basis, however Different explanations for objective improvement of the physical work capacity are con ceivable, e g. increased myocardial performance (increased stroke volume in relation to the ha modynamic prerequisites), decreased work for the right or left ventricle per circulatory ach evement (mainly through reduced pulmonary or peripheral resist ance and possibly also through a change in the neurohumoral control of the myocardium), a reduced t ndency to disturbing arrhythmias and, finally a change of the metabolic control during muscular work.

Studies on the effect of physical training on the central and peripheral circulation in patients with coronary heart disease have been sparse and have only comprised a few patients, usually with no control group (16 26, 27 70) Mostly a decrease in the work of the heart in relation to the external work performed has been observed after training. Some studies (16, 17) have been concentrated mainly on effects on the peripheral circulation, while in others (25 26 27) more attention has been paid to changes in the central circulation

The general aim of the present study was to compare the circulatory function after a period of physical training n part of a series of consecutive patients with myocardial infarction, with that in a reference group from the same series. These patients have usually an abnormal relationship between intracardiac pressure and flow Firstly an attempt was made to find out if at least partial normalization of this relationship could be obtained. Secondly a study was made of the nfluence of age on the degree of measurabl ha modynamic changes, and thirdly th question of whether the training mainly affected the central or the peripheral circulation was investigated

A preliminary report of this paper was presented at the European Congress of Cardiology in Madrid, September 26th 1972.

ABSTRACT

The study was made on 63 male patients treated at the University Hospital, Uppsala, for acute myocardial infarction, with an onset during the period 15/3 1969—15/3 1972.

Patients with the following were excluded previous cardiac infarction, a diastolic blood pressure at rest of more than 110 mm Hg· a relative heart volume of more than 600 ml manifest diabetes mellitus; other complicating diseases, e.g. pulmonary disease or arthrosis of the hips or back. All patients equal to or more than 70 years of age were also excluded.

The 63 patients selected were admitted t hospital 3 months after the onset of the infarction for examination including right heart catheterization. They were then classified randomly to trained (34 patients) or reference group (29 patients).

Starting 3 months after the onset of the infarction, the patients of the trained group underwent a 3-month period of individual physical training (3 one-hour sessions per week under th leadership of physiotherapist). The patients of the reference group carried out only their daily physical activity during this period (the reference period). Six months after th onset of the infarction the patients of both groups were readmitted t hospital for second examination including heart catheterization.

During th approximately 1000 training sessions no arrhythmias or other serious complications occurred The emergency equipment that was always available (including defibrillator) was never eeded.

The haemodynamic investigation showed that during work the pressure in the pulmonary circulation, including the PCV pressure, was pathologically increased. N change after the training period was noted. I both the trained and reference group lower heart rat at rest was recorded after the training or reference period. During work a decrease was noted in both groups, but this was more marked in the trained group. The stroke volume during submaximal work increased in the trained group, especially in the younger patients without angina pectoris. The cardiac output at rest and during work remained unchanged in both the trained and reference group. Neither was there any alteration of the arteriovenous oxyge difference Th left ventricular minut work was decreased after the training due to a reduction of th arterial blood pressure during exercise. The reference group showed no significant reductions of left entricular minut work or blood pressure Finally the peripheral and entral circulatory effects of the training, and their possible underlying mechanisms, were discussed.

From both groups there was a considerable drop out amounting to a total of 21 out of 63 patients (13 in th trained group and 8 in the reference group). One patient (case 5) was cluded from the trained group because of lack of interest Three patients (cases 6, 17 and 20) were excluded from the trained group and 4 (cases 6 12, 16 and 26) from the reference group because they did not wish to undergo a second catheterization. None of the patients refused the first catheterization. In the trained group one reinfarc tion occurred (case 9), and another patient from that group (case .8) ollapsed at home and died within th course of a few minutes, probably of infarction. One patient in the reference group (case 11) was x cluded during the reference period (for definition see p. 9) because of increasing hypertension, which required active treatment with beta receptor block ng agents. One patient (case 24) was excluded from the trained group owing to an onset of ulcerative proctitis, which needed medical treatment and rest. Th ee patients (cases 11 12 and 16), who were over 60 years old, were excluded from the trained group on ccount of deterioration with increasing severity of angina pectoris case 16 also began to have breathlessness at rest. Two patients (cases 12 and 13) also developed pain in the hip joints and back. One patient (case 15) sustained a rupture of a thigh muscle at the first training session, but this only necessitated a two-week break in the training.

Three pat ents in both groups were excluded for technical reasons. During th Fick analysis in one patient in each group leakage was noted on collec tion of expired air Case 3 n th trained group was excluded because at the second catheterization he disco tinued his work before the work period was completed

In one patient in the trained group (case 14) and 3 patients in the referenc group (cases 5, 9 and 18) th catheter could not be advanced t th pulmonary art ry and these patients were therefore also ex l ded from th calculations of th results, e cept case 5 for whom the cardiac output was calculated with the tip of the catheter lying in th right trium at both catheterizations The risk of error in the F ck ana lysis is somewhat greater with this catheter positio (66) In 3 patients in th trained group (cases 2, 8 and .3) and in one patient in the reference group (case 8) the attempt to puncture the peripheral artery failed I these patients th cardiac output was calculated on the assumption that the o y g n saturation both at rest nd during work was 97

Table I. Age height and weight in the trained and reference group after exclusions. There was a significant difference in height between the younger and older patients in the reference group (P<0 001).

		Age (yrs)	Height (cm)	Weight (kg)
TRAINED GROUP				
T tal	Mean	52.5	175	76.3
	SEM	1 75	1 49	1.69
	Range	35-68	164-195	64.0-91.5
	N	21	21	21
<55 yrs	Mean	48 4	176	76.8
	SEM	1.54	1.88	2.25
	Range	35-55	164-195	65.1-91.5
	N	14	14	14
>55 yrs	Mean	60.9	173	75.2
	SEM	1.67	2.49	2 49
	Range	56-68	165 183	64.0-80.5
	N	7	7	7
REFERENCE GROUP				
Total	Mean	55.5	174	76 7
	SEM	2.02	1.14	2.13
	Range	36-69	165 184	60.6-98.0
	N	21	21	21
<55 yrs	Mean	47.9	178	77 7
	SEM	2.13	1 49	3 49
	Range	36-55	168-184	65.8-98.0
	N	10	10	10
>55 yrs	Mean	62.5	171	75.8
	SEM	1.34	1.05	2.68
	Range	56-69	165 175	60.6-90.0
	N	11	11	11

None of the patients were being treated with digitalis or beta receptor blocking agents during the training or eference period. After the exclusions there wer 8 smokers n the trained group and 10 in the reference group

In *Table I* the age height and w ght of th trained and reference groups are presented after the above exclusions The two main groups were each divided into 2 bgroups those 55 years of age or younger and those older than 55 years Th final trained group the average 3 years younger than the f r e g p but this difference was not significant A be seen in the tabl ther were no sign fic d fferences between th main and subgroups w th espe t to h ight and weight, except be tw th f rence subgroups. The number of patient h h main groups was 21

The work tests were performed according to the method of Sjöstrand (64) and Wahlund (69) with an electrically braked ergometer bicycle (41), and with continuous ECG recording.

Training model

The patients of the reference group were given no instructions about increased physical activity or training they were advised to take a daily walk, which should be increased gradually This was in accordance with the routine followed in this hospital before 1969 The possibility that a reference patient might be told about the training by a patient in the trained group on meeting in hospital waiting rooms, for example, could hardly be prevented or excluded but on no occasion did a reference patient ask to take part in the training programme

The physical training was carried out 3 times a week at the hospital Each training session was led by a qualified physiotherapist and lasted about 1 hour Each patient was trained individually as the number of patients was too small to form a group

Figure 3 shows the schematic arrangement of a training session. It started with 10 min rest, and this was followed by 10 min of calisthenics under the leadership of the physiotherapist, and then 3 ten-minute sessions of cycling on an ergometer bicycle with varying work loads Between each cycling session the patient rested, sitting on the bicycle, for 3 min. The training ended with 10 min rest on a couch. The patient was then allowed to wash his head and arms, but showering was not advised It was considered desirable that the work load should reach but not exceed the pain threshold for the patients with angina pectoris. The highest heart rate in the training sessions towards the end of the training period was, on the average 148±(SEM) 2 beats/minute for the younger patients (≤55 years) and 133±4 for the older patients (>55 years). The difference between these two groups was statistically significant (P<0 001).

Of the 16 patients *without* angina pectoris in the trained group only 2 had chest pain during the training sessions viz. case 26 (68 years old), who had pain in the chest on one occasion, and case 5 (45 years old), whose chest pain was associated with mild arrhythmia (3—4 isolated ventricular extrasystoles). The tendency to arrhythmia disappeared when a malfunctioning on-demand pacemaker had been removed. This patient then discontinued the training because of lack of interest.

Of the 15 patients in the trained group *with* angina pectoris the pain threshold was not reached in 2 cases in case 13 there was a tendency to arrhythmia and despite a typical history of angina in case 34 and a heart rate of about 150 beats/minute during the training, this patient did not have chest pain during the training sessions either This was possibly due to the fact that before each training session he had to walk up a slight hill to the hospital from the railway station. During this walk he almost always had a typical attack of angina pectoris. At the subsequent

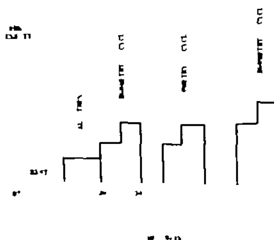


Fig. 3 Training design.

training session indoors after having been "warmed up" he therefore had no pain (second wind phenomenon). Because of severe angina pectoris the training was regarded as inadequate in case 27 in whom the highest heart rate during the training sessions did not e ceed 130 beats/minute

The training period comprised about 30 sessions. At each session the physiotherapist filled in a record card where she noted the carotid or radial puls and the indirect brachial arterial pressure The heart rate and blood pressure were recorded before and after the session and at the end of each 10 minute cycling period. Any unfavourabl reaction, such as ar rhythmia or congestion and d creasing blood pressu re was also noted A physician was always available in an adjacent room, where also emergency equipment including an ECG apparatus and a defibrillator were kept in readiness.

The patients were not connected to an oscilloscope during the training sessions

Comments

In the approximately 1000 training sessions o serious complications such as reinfarction, cardiac arrest or tachyarrhythmias occurred Thus the defibrillator never needed to be used, nor any of the other emergency equipment. The 2 cases of re infarction did not occur during training, but in both cases about 24 hours after the last training session during this session no complications were noted.

The pat ents showed very positive attitude towards the physical training project as form of treat ment Only one patient (case 5) lacked interest and did not attend the training sessions regularly

In 2 patients (cases 6 and 27) th training was considered to be inadequate from a somatic point of view as the heart rat was kept too low during the training sessions for safety reasons.

Procedure for right heart catheterization

The day before th cathet rizationth patient isited th heart laboratory where h was told i detail about th procedur f th examination on th following d y After fasting overnight h was give 0 1 amobarb tal, accord ng to the routine n hour before th xaminat on in the morning

I 76 examinations 70 mm long teflon catheter (I fart 1 15 mm, St lle Werner Stockholm) was int oduced percutaneously into the radial artery I 70 examinations the brachial artery was used instead, in which case a short polyethylene catheter (PE 160) was introduced by the Seldinger technique (63). Right heart catheterization was then performed in the usual way: the catheter was introduced through a small incision in a superficial medial cubital vein and was advanced to the pulmonary artery

After the insertion of the 2 catheters and measurement of the pulmonary capillary venous (PCV) pressure the patient rested for 10 min. The cardiac output (CO) at rest was then determined by the direct Fick principle Expired air was collected in a Douglas bag for 10 min (2.), after flushing of the bag and the tube system with expired air for a few min. The heart rate was noted every other min from the ECG and the pressure in th systemic artery (SA) and the pulmonary artery (PA) was oted. Between the 5th and the 6th min blood samples were taken simultaneously from SA and PA for measurement of the arteriov nous oxygen difference (AVD_{O_2}).

When th tip of the catheter had been placed in the PCV position, CO was determined during exercise, the patient cycling in the supine position for 6 min with the feet strapped to an electrically braked bicycle ergometer (Elema Schönander Stockholm). The mean work load was 320 kpm/min (52 W) (range 100–500 kpm/min) for the trained group and 296 kpm/min (49 W) (range 150–500 kpm/min) for the reference group. This difference was not significant. The work load was set at approximately half of the highest attained load at the work tests of the previous days. ECG and intra ascular pressures were recorded continuously on direct writing recorder (Mingograf 81).

The exp red air was collected during the last 3 min of the xamination During the first 3 min the tip of th cath ter was in the PCV position, between the 3rd and 4th min it was withdrawn to PA and be twee the 4th and 5th min blood samples were taken simultaneously from PA and SA for determination of AVD_{O_2}

Sev patients had chest pain d ring xercise during the catheterization procedure, 3 of them at both examinations

At th second heart catheterization each patient had th sam work load as at the f rst catheteriza t on

Comments

N sev re complications, such as entricular fibrilla t on occurred during the heart catheterizations, and

neither was there any attack of ventricular tachycardia. On the other hand, 2 cases in the reference group (cases 2 and 18) showed transient atrial fibrillation which regressed after i v administration of 0 4 mg Cedilanid.® Both of these attacks of atrial fibrillation occurred when the tip of the catheter was in the right atrium. An impression was obtained that these post infarction patients did not have a greater tendency to extrasystoles during the manipulations of the catheter than patients with other cardiac diseases.

As mentioned above in 76 of the catheterizations the radial artery was used and in 20 the brachial artery In 14 cases different arteries were used at the two examinations. In 5 of these cases (including 2 from the trained group) a higher blood pressure (>5 mmHg) was noted in the radial artery in 4 cases (including 2 from the trained group) the blood pressure was higher in the brachial artery and in 5 cases (including one from the trained group) the blood pressures in the two arteries were the same. It was therefore considered of no importance for measurement of the blood pressure which of these arteries was used.

For measurement of CO the direct Fick method was used The volume of expired air was measured with a gasometer (Nordgas Stockholm) and the ired air was analysed according to the method of ldane with some modifications (21) Duplicate terminations were performed in the gas analyses and the error of a single determination in this laboratory was 0 02 vol% for oxygen and 0 01 vol% for carbon dioxide. The values for *oxygen uptake* (V) were compared with the normal values obtained from Harris & Benedict (32).

The *oxygen saturation* was determined spectrophotometrically on haemolysed whole blood (43 44) the error of a single determination was 0.2 / with an oxygen saturat on of about 75 / The *haemoglobin concentration* was measured spectrophotometrically after conversion to cyanmethaemoglobin The error of a singl determination was 0 02 g% The *ygen capacity* was determined from the haemoglob n concentration under the assumption of an o yge -binding capacity of 1 39 ml O_2/g Hb (19 67). The *oxygen content* was obta ed from the values for oxygen saturat on and oxygen capacity with the addition of physically dissol ed o ygen AVD_0 is the differen n o yg ontent in th blood between SA and PA

Fick s equation gives the rdia tput through the formula. *oxygen uptake* ml/min = *cardiac output* l/min x *arteriovenous oxygen difference* ml/l

Wranne (73) found in this laboratory an error of 6 1 / in a determination by the direct Fick method, obtained in duplicate determinations of the cardiac output in 10 cases

The *cardiac index* (CI) (l/min·m²) was calculated by dividing cardiac output by body surface area as obtained from Du Bois & Du Bois (18)

Pressures were measured by pressure transducers of the capacitance type (EMT 34 or 35 Elema Schönander Stockholm). These were connected to amplifier units and an electromanometer (EMT 31 Elema-Schönander Stockholm) The pressure curves and ECG were recorded on an eight-channel Mingograf 81 and the mean pressures were obtained by electrical integration The paper speed was 25 mm per sec except when the end-diastolic pressure of the right ventricle was measured when it was 50 mm/sec

The reference level for zero pressure in the supine position was placed 5 cm dorsal to the sternal insertion for the fourth rib Calibrations were performed with a water manometer before each examination

The *pulmonary vascular resistance* (PVR) was calculated from the ratio

$$\frac{PA_m - PCV_m}{CO}$$

and expressed in mm Hg/l/min CO = cardiac output, PA_m = pulmonary arterial mean pressure, PCV_m = mean pulmonary capillary venous pressure.

The *left ventricular minute work* (LVMW) (kpm/min) was calculated from the formula

$$LVMW = \dot{Q} \times MABP \times 0.0144 \text{ kpm/min}$$

where $\dot{Q}$ = cardiac output, MABP = mean arterial blood pressure and 0 0144 = the product of the specific density of blood, 1.056 g/ml, and that of mercury 13 6 g/ml (10), divided by 1000 to convert mm into m.

The *left ventricular stroke work* (LVSW) was calculated from the formula

$$LVSW = SV \times MABP \times 0\,0144 \text{ g·m}$$

where SV = stroke volume (ml)

Statistical methods

(n collaboration with Bernhard Hultfeldt)

A General concepts and definitions.

Th following def nitions of statistical concepts have b n used

i) Mean $M = \frac{1}{N} \Sigma x_i$ N = number of observations.

ii) Variance VAR = $\frac{1}{N-1} \Sigma(x_i - M)^2$

iii) Standard deviation SD = $\sqrt{VAR}$

iv) Standard error of the mean SEM = $\frac{SD}{\sqrt{N}}$

v) Slope $\frac{y_2 - y_1}{x_2 - x_1}$

B. Statistical analysis of the difference between values at rest and during work.

i) The change in individual slope

Bivariate observations were made concerning various physiological variables, e.g. stroke volume and oxygen uptake. This was done both before and after the training or reference period and in each instance both at rest and during work. Individual slopes were calculated, assuming one of the variables to be the dependent one (see Fig. 8). The mean and standard deviation of the differences between individual final and initial slopes were then calculated both in the trained and in the reference group. Each of the means was tested for significance by a t test for paired differences described in any statistical textbook (37). This gave an indication of whether any change had occurred in the relationship between the two studied variables from the first examination to the second. With the two variables mentioned above as an example, the question was whether for a given oxygen uptake (amount of work) the stroke volume increased on the average between the two examinations.

ii) Comparison between trained and reference group

To find out whether the trained group had gained more in physiological capacity during the intervening period, the means of the slope differences in the two groups were compared in a test of significance. The method used was Student's t-test for differences in independent means.

C. Statistical analysis of the differences between values after and before the training or reference period.

The effect of training was analysed by studying the difference in mean change for different variables in two groups. This was done in a multiple regression analysis with an indicator variable for classification group. Values before training, age, place of residence (urban/rural), and occurrence of angina pectoris were used as other independent variables. This procedure makes the comparison relatively efficient as influences on the differences in mean changes from those variables mentioned above will be eliminated. The coefficient for the indicator variable was tested for significance by means of Student's t-test.

D. In other comparisons between the trained and reference group Student's t-test was used, but for comparisons of changes within each group and for each patient only a t test for paired differences.

E. Significance levels.

The term significant is used in accordance with the following convention. All test are tested at the 5 % level of significance. If an observed deviation from the null hypothesis is of such a magnitude that the probability P of obtaining a deviation at least as great as the observed one is greater than 0.05 (here the null hypothesis is assumed to hold), then the observed deviation is consequently said to be non-significant and is designated ns. If P is less than 0.05 the deviation is said to be significant.

The degree of significance has been given, using the following notation.

If $0.001 < P < 0.05$ the significance is denoted by $P < 0.05$

If $0.001 < P < 0.01$ the significance is denoted by $P < 0.01$

If $P < 0.001$ the significance is denoted by $P < 0.001$

RESULTS

In the following values obtained before the training or reference period are called initial values and those obtained after those periods are called final values.

Circulation at rest

In *Table II* some data are given at rest. The mean *ventilation* was normal in both groups and there were no significant differences either before or after the training or reference period. Case 21 in the trained group had considerably increased values on both occasions, 17.8 and 11 9 l/min. The reason for this is not clear but his oxygen uptake was also somewhat high However there were no signs of hyper thyroidism or pronounced anxiety Case 31 in the trained group and cases 3 and 24 in the reference group had values e ceeding 10 l/min both before and after the train ng and reference period These high values in relation to the others were considered to be due to apprehension

The initial *oxygen uptake* was 245±(SEM) 9 ml/min or 6 above the predicted basal oxygen uptake in th trained group The corresponding value for the reference group was 240±8 ml/min or 4 % above the predicted basal value The final values showed no significant changes and there were no significant differences between the age groups

The mean *arterial oxygen saturation* was normal in all groups nd there were no significant differences between initial and final alues. In the trained group one patient (case 1) with no manifest pulmonary disease or signs of right to left shunt had a alue of 91.8 at the first examination His alue after training was 94 6

The *arteriovenous oxygen difference* showed no significant changes after the trai ing or reference period. No significant differences were found between either the main groups or the age groups Four patients in the trained group and tw patients the reference group had values exceeding 60 ml l Three of these patients were older than 60 ears or had angina pectoris.

The mean *cardiac output* showed no significant changes after the training or reference period. In both main groups the younger patients had larger values than the older ones the difference was significant in the trained group for both the initial values ($P<0.02$) and the final ones ($P<0.05$) This difference was due to the higher V_{O_2} and lower AVD_{O_2} in the younger patients. Two older patients in the trained group (cases 6 and 26) had values of 3.0 l/min or lower

The *cardiac index* reflected the cardiac output, but there were no significant differences in this respect

From *Table III* it can be seen that the mean *stroke volume* was not significantly changed in the trained or reference group In both groups there was a tenden cy to lower values in the older patients The final value for the older reference patients was, however significantly higher than the initial one

The mean *heart rate* lay between 50 and 90 beats/min in all patients. Both in the trained and reference group there was a significant decrease from initial to final values. The changes were also significant in the age groups except for the older patients in the trained group, for whom the final value was the same as the initial one.

The mean *pulmonary vascular resistance* was normal both before and after the training or reference period. There were no significant changes or differences

The *left ventricular minute work* decreased in both main groups after the training or reference period, but the changes were not significant Neither were there any significant changes in *left entricular stroke ork* except in the older patients of the reference group, where the initial value was 97±8 and the final alue 118±5 g m ($P<0.05$).

Pressures

Table II gives the *pressures* in the right heart and the PCV position All recorded pressures lay within normal limits There w re no significant changes except for the older patients in the trained group, in whom the PCV pressure decreased from 8±0.8 to 5±0.3 mm Hg

Table II *Haemodynamic flow data at rest before and after the physical training or reference period (A).*

		Ventilation (l/min)		Oxygen uptake (ml/min)		Arterial oxygen saturation (%)		Arterio-venous oxygen diff. (ml/l)		Cardiac output (l/min)		Cardiac index (l/min·m^2)	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP													
Total	Mean	8.2	7.8	245	239	96.3	96.6	47.5	49.3	5.3	4.9	2.7	2.6
	SEM	0.56	0.31	9.39	7.52	0.43	0.41	1.33	1.01	0.22	0.18	0.12	0.10
	Range	4.9-17.8	5.3-11.9	156-384	186-335	91.8-99.9	93.9-99.9	37.7-68.0	41.2-60.9	3.0-8.2	3.4-7.3	1.7-4.4	2.0-3.8
	N	22		22		20		23		22		21	
≤55 yrs	Mean	8.5	8.0	255	243	95.8	96.8	46.5	49.2	5.6	5.0	2.9	2.6
	SEM	0.78	0.37	11.7	6.89	0.52	0.52	1.74	1.38	0.26	0.15	0.14	0.09
	Range	5.2-17.8	6.3-11.9	197-384	200-290	91.8-99.9	93.9-99.9	37.7-68.0	41.2-60.9	4.2-8.2	4.1-5.7	2.4-4.4	2.1-3.2
	N	15		15		14		16		15		15	
										$P < 0.02$	$P < 0.05$		
>55 yrs	Mean	7.7	7.3	224	229	97.4	95.9	49.8	49.3	4.5	4.7	2.4	2.5
	SEM	0.57	0.57	13.5	19.1	0.65	0.64	1.58	1.14	0.29	0.48	0.17	0.28
	Range	4.9-9.4	5.3-9.7	156-256	186-335	95.6-99.2	94.4-98.8	44.1-54.3	44.4-52.1	3.0-5.4	3.4-7.3	1.7-2.8	2.0-3.8
	N	7		7		6		7		7		6	
REFERENCE GROUP													
Total	Mean	9.0	8.7	240	239	96.2	96.5	48.6	49.3	5.1	4.9	2.7	2.6
	SEM	0.40	0.42	7.97	7.33	0.41	0.32	1.43	1.12	0.28	0.17	0.12	0.10
	Range	6.4-13.4	6.3-13.8	169-301	188-318	92.1-99.5	93.1-98.7	34.6-63.0	38.8-57.4	3.1-8.4	3.8-6.6	1.7-4.4	2.0-3.8
	N	21		21		21		21		21		21	
≤55 yrs	Mean	9.3	8.7	253	246	96.6	96.9	47.4	49.9	5.5	4.9	2.9	2.6
	SEM	0.71	0.53	12.1	11.3	0.46	0.28	1.95	1.27	0.44	0.25	0.14	0.09
	Range	6.4-13.4	6.6-11.0	188-301	188-318	94.5-98.8	95.6-98.4	34.6-54.4	43.2-57.4	3.7-8.4	4.0-6.6	2.4-4.4	2.1-3.2
	N	10		10		10		10		10		10	
>55 yrs	Mean	8.7	8.7	228	232	95.9	96.1	49.8	48.5	4.7	4.8	2.4	2.5
	SEM	0.44	0.66	9.70	9.58	0.67	0.55	2.10	1.85	0.34	0.24	0.14	0.09
	Range	6.9-11.0	6.3-13.8	169-271	199-306	92.1-99.5	93.1-98.7	37.1-63.0	38.8-56.0	3.1-7.1	3.8-5.9	1.7-2.8	2.0-3.8
	N	11		11		11		11		11		11	

Table III. Haemodynamic flow data at rest before and after physical training or reference period (B)

		Stroke volume (ml)		Heart rate (beats/min)		Pulmonary vasc. resist. (mm Hg/l/min)		Left ventricular minute work (kpm/min)		Left ventricular stroke work (g·m)	
		Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP											
Total	Mean	77	77	67 P<0.001	64	1.0	1.2	7.5	6.9	110	109
	SEM	3.30	3.09	1.82	1.73	0.12	0.13	0.46	0.39	6.25	5.13
	Range	48-110	58-110	52-85	49-77	0.2 2.4	0.5 2.9	3.9-12.7	4.7 11.1	81 177	78-154
	N	22		23		21		18		18	
≤55 yrs	Mean	80	80	68 P<0.001	63	1.0	1.0	8.1	7.2	117	116
	SEM	4.28	3.62	2.41	2.41	0.14	0.12	0.57	0.49	7.38	5.74
	Range	58 110	60-110	52-85	49-77	0.2 2.1	0.5 2.1	6.0-12.7	5.3-11.1	89-177	88-154
	N	15		16		14		12		12	
>55 yrs	Mean	70	71	65	65	1.2	1.6	6.3	6.3	97	95
	SEM	4.57	5.58	2.14	1.91	0.22	0.26	0.54	0.62	10.3	7.97
	Range	48-84	58 101	54-71	59 72	0.7 2.4	0.8-2.9	3.9-7.8	4.7-8.4	81 130	78-116
	N	7		7		7		6		6	
REFERENCE GROUP											
Total	Mean	72	76	70 P<0.001	65	1.1	1.2	7.6	7.3	107	115
	SEM	3.51	2.55	1.87	1.69	0.13	0.16	0.60	0.31	7.74	5.05
	Range	49 111	53-95	53-88	46-78	0.2 2.0	0.0-2.7	4.0-14.7	5.3-10.7	60-210	78-178
	N	21		21		20		19		19	
≤55 yrs	Mean	77	75	71 P<0.05	67	1.0	1.2	8.5	7.3	118	111
	SEM	5.51	4.10	2.81	2.33	0.18	0.19	0.98	0.33	13.1	9.59
	Range	63-111	53-95	60-88	57 76	0.3-1.9	0.5 2.3	5.3-14.7	5.3-10.7	82 201	78-178
	N	10		10		10		9		9	
>55 yrs	Mean	68 P<0.05	77	70 P<0.01	63	1.3	1.1	6.8	7.3	97 P<0.01	118
	SEM	4.21	3.33	2.58	2.39	0.17	0.27	0.67	0.37	8.07	4.62
	Range	49-92	57-93	53-84	46-78	0.2 2.0	0.0-2.7	4.0-11.8	5.5-9.0	60-152	82 133
	N	11		11		10		10		10	

Table IV Haemodynamic pressure data (mm Hg) at rest before and after the physical training or reference period (4). There was significant difference (P>0.05) between the final PCV values of the two older groups.

		RA Mean		RV Systolic		RV End-diastolic		PCV Mean	
		Before	After	Before	After	Before	After	Before	After
TRAINED GROUP									
Total	Mean	3	3	23	24	3	3	8	7
	SEM	0.38	0.38	0.78	0.74	0.45	0.46	0.57	0.44
	Range	–2 7	0-7	18-36	16-30	–2 7	–2 7	5-16	4-11
	N	23		23		23		22	
<55 yrs	Mean	3	3	23	24	3	3	8	8
	SEM	0.45	0.48	0.58	0.77	0.57	0.61	0.78	0.47
	Range	–2-6	0-6	19-26	16-30	–2 7	–2 7	5-16	4-11
	N	16		16		16		15	
>55 yrs	Mean	4	4	24	23	4	3	8 P<0.01	5
	SEM	0.48	0.59	2.25	1.74	0.40	0.64	0.71	0.25
	Range	3-7	2 7	18-36	16-29	3-6	1-6	6-28	4-28
	N	7		7		7		7	
REFERENCE GROUP									
Total	Mean	2	2	22	24	3	3	7	7
	SEM	0.33	0.40	1.11	0.89	0.50	0.25	0.62	0.41
	Range	–1-4	0-6	16-36	17 34	–2-6	–2-6	1 11	4-10
	N	21		20		19		20	
<55 yrs	Mean	2	3	22	23	3	3	7	6
	SEM	0.47	0.51	1.37	0.99	0.64	0.37	0.53	0.54
	Range	–1-4	0-6	16-28	17 27	0-5	2 5	6-9	4-10
	N	10		10		9		10	
>55 yrs	Mean	2	2	23	25	3	3	7	7
	SEM	0.48	0.38	1.80	1.43	0.77	0.34	1.16	0.62
	Range	–1-4	0-4	18-36	20-34	–2-6	2-5	1-11	4-10
	N	11		10		10		10	

(P<0.01). There was also a significant difference bet een the final alues in the two older age groups (P<0.05).

Table V present the pressures n the *pulmonary* and s *stemic arteries*. All pulmonary arterial pressures lay within normal limits and there ere no significant changes or differences. The same pplies t the ystemic artery xcept for the mean pressure in the older patients of the reference group, which rose f om 97+2 t 107 3 mm Hg (P<0.05). The final systolic and mean pres ures of the trained group were signif cantly lower than the corresponding pressures of the reference group due to differences between the older ge group

Table V Haemodynamic pressure data (mm Hg) at rest before and after physical training or reference period (B). After the training or reference period there was a significant difference (P<0.02) in the systolic SA pressure between the two main groups. Also the systolic pressure in the older age groups was significantly different (P<0.01). There was also a significant difference (P<0.05) in mean pressure in the two main groups after the training or reference period.

		Pulmonary artery						Systemic artery					
		Systolic		Mean		Diastolic		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP													
Total	Mean	22	21	13	13	8	8	136	133	97	96	73	73
	SEM	0.85	0.81	0.55	0.58	0.51	0.51	4.51	3.92	3.55	3.40	13.2	11.8
	Range	15 29	15 28	8-19	9 20	2 14	5 14	110-195	110-170	80-150	80-135	60-110	60-100
	N	23		23		23		19		19		19	
<55 yrs	Mean	21	21	13	13	8	8	136	135	97	98	73	74
	SEM	1.06	0.95	0.63	0.71	0.56	0.63	6.18	5.30	4.99	4.55	3.65	3.73
	Range	15 29	15 27	8-18	9-20	2 12	5 14	110-195	110-170	80-150	80-135	60-110	60-100
	N	16		16		16		13		13		13	
>55 yrs	Mean	22	21	13	12	8	7	135	130	96	92	73	72
	SEM	1.49	1.62	1.15	0.92	1.10	0.81	5.79	5.18	3.75	4.22	1.67	3.34
	Range	16-28	17 28	10-19	10-16	6-14	5 10	120-155	110-145	85-110	80-105	70-80	60-80
	N	7		7		7		6		6		6	
REFERENCE GROUP													
Total	Mean	20	20	12	13	7	7	138	148	101	105	76	78
	SEM	1.13	1.09	0.71	0.70	0.47	0.38	4.76	4.41	2.97	2.56	3.09	2.13
	Range	12 29	13-30	6-18	9-19	5-10	5 11	100-210	120-185	90-150	90-130	60-100	65-100
	N	20		19		20		20		20		20	
<55 yrs	Mean	19	20	12	13	7	7	136	137	104	101	79	80
	SEM	1.37	1.55	0.83	1.13	0.48	0.51	9.41	6.56	5.97	4.14	3.09	3.37
	Range	12 27	13-25	9-15	9-18	5-9	5-11	120-210	120-185	95 150	90-130	70-100	70-100
	N	10		9		10		9		9		9	
>55 yrs	Mean	22	21	12	13	7	8	139	156	97 P<0.05	107	73	76
	SEM	1.74	1.56	1.16	0.92	0.82	0.57	4.48	4.81	2.17	3.12	2.16	2.77
	Range	16-29	17 30	6-18	10-19	5-10	5-11	100-160	130-185	90-115	95-120	60-85	65-95
	N	10		10		10		11		11		11	

Circulation during exercise

The ventilation (Table VI) decreased significantly in both main groups after the training or reference period. In th reference group there was also a significant decrease in the younger age group.

The oxygen uptake during exercise represented an approximately four-fold increase from the resting

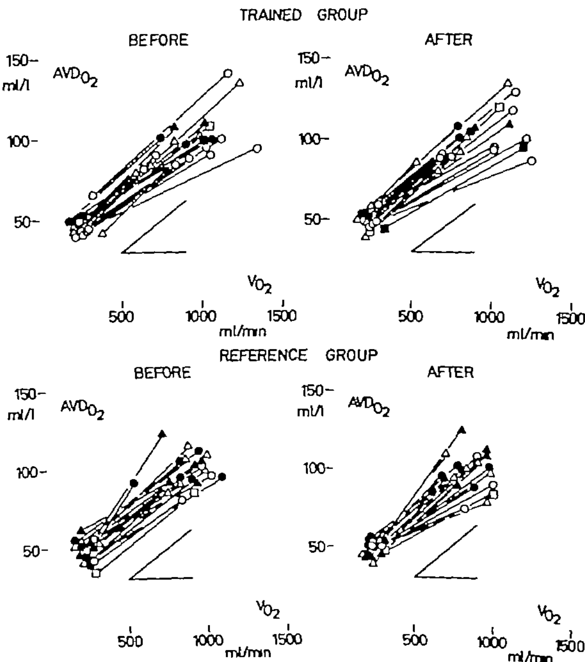


Fig. 4. Arteriovenous oxygen difference (AVD_{O_2}) in relation to oxygen uptake (V_{O_2}) at rest and during ork in the supine position for 21 trained and 21 reference patients before and after the training or reference period.
Open symbols represent patients ≤ 55 years of age, and *filled* symbols patients > 55 years of age. *Triangles* represent patients with angina pectoris and squares patients with questionable angina pectoris. *Circles* represent patients without angina pectoris.
The mean slope of the line rest ork is indicated in each of the four sub figures.

Table V *Haemodynamic pressure data (mm Hg) at rest before and after physical training or reference period (B). After the training or reference period there was significant difference (P<0 02) in the systolic SA pressure between the two main groups Also the systolic pressure in the older age groups was significantly different (P<0 01). There was also significant difference (P<0 05) in mea pressure in the two main groups after the traini g or reference period.*

		Pulmonary artery						Systemic artery					
		Systolic		Mean		Diastolic		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP													
Total	Mea	22	21	13	13	8	8	136	133	97	96	73	73
	SEM	0 85	0 81	0.55	0.58	0.51	0.51	4.51	3.92	3.55	3 40	13.2	11.8
	Range	15 29	15 28	8 19	9 20	2 14	5-14	110-195	110-170	80-150	80-135	60-110	60-100
	N	23		23		23		19		19		19	
<55 yrs	Mean	21	21	13	13	8	8	136	135	97	98	73	74
	SEM	1.06	0.95	0.63	0 71	0.56	0.63	6 18	5.30	4.99	4.55	3.65	3 73
	Range	15-29	15-27	8-18	9-20	2 12	5 14	110-195	110-170	80-150	80-135	60-110	60-100
	N	16		16		16		13		13		13	
>55 yrs	Mean	22	21	13	12	8	7	135	130	96	92	73	72
	SEM	1 49	1.62	1 15	0.92	1 10	0.81	5 79	5 18	3 75	4.22	1.67	3.34
	Range	16-28	17 28	10-19	10-16	6-14	5 10	120-155	110-145	85-110	80-105	70-80	60-80
	N	7		7		7		6		6		6	
REFERENCE GROUP													
Total	Mean	20	20	12	13	7	7	138	148	101	105	76	78
	SEM	1 13	1.09	0 71	0 70	0 47	0.38	4 76	4 41	2.97	2.56	3.09	2 13
	Range	12 29	13-30	6-18	9 19	5-10	5 11	100-210	120-185	90-150	90-130	60-100	65-100
	N	20		19		20		20		20		20	
<55 yrs	Mean	19	20	12	13	7	7	136	137	104	101	79	80
	SEM	1.37	1.55	0.83	1 13	0 48	0.51	9 41	6.56	5.97	4 14	3.09	3.37
	Range	12-27	13-25	9-15	9 18	5-9	5-11	120-210	120-185	95-150	90-130	70-100	70-100
	N	10		9		10		9		9		9	
>55 yrs	Mean	22	21	12	13	7	8	139	156	97 P<0 05	107	73	76
	SEM	1 74	1.56	1 16	0.92	0.82	0.57	4 48	4.81	2.17	3.12	2.16	2.77
	Range	16-29	17 30	6-18	10-19	5 10	5-11	100-160	130-185	90-115	95-120	60-85	65-95
	N	10		10		10		11		11		11	

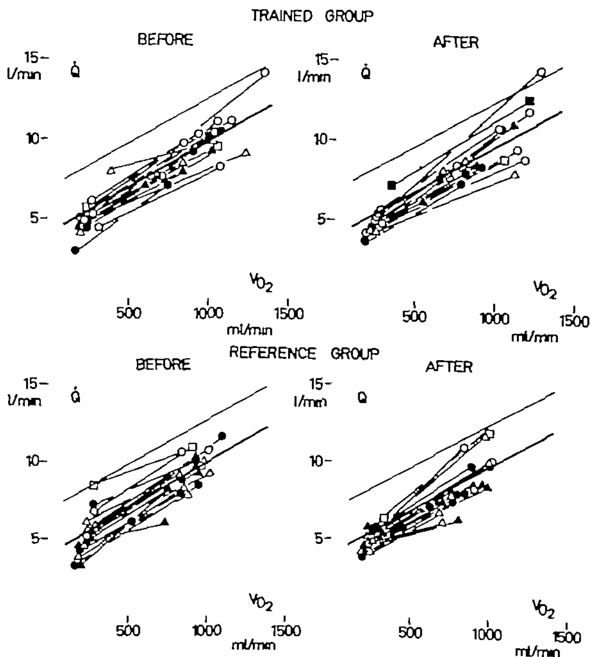


Fig. 5 Cardiac output (Q̇) in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest and during work in the supine position for 1 trained and 21 reference patients before and after the training or reference period. Symbols as in Figure 4. The *heavy* line represents the regression line during exercise for healthy men aged 61–83 (Granath *et al.* (29)) and the *thin* line the corresponding regression line for healthy men aged 23–41 (Holmgren *et al.* (40)).

alue. There w r no significant ch nges after th training or reference period or differences between groups. The younger patients of both groups had a higher oxygen uptake than the older ones.

Th *arterial oxygen saturation* was normal and remained essentially unchanged after the trai ing or reference period in th two groups. Also in the subgroups small and n ignif cant changes were noted. The m an *arteriovenous oxygen difference* showed no significant changes after the training or referenc period. Neither were there any significant differences betw en th main or age groups. *Figure 4*

TRAINED GROUP

REFERENCE GROUP

Q

l/min

AFTER

BEFORE

Fig 6. Cardiac output (Q) during work before and after the training or reference period in 21 trained and 21 reference patients. Symbols as in Figure 4

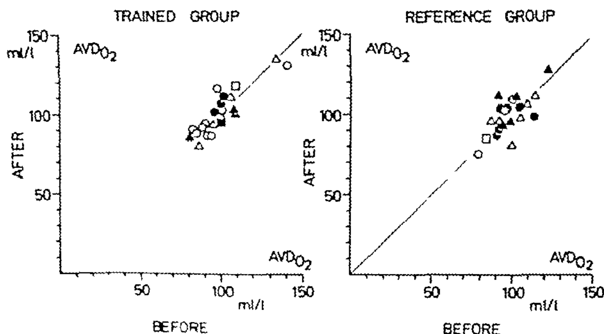
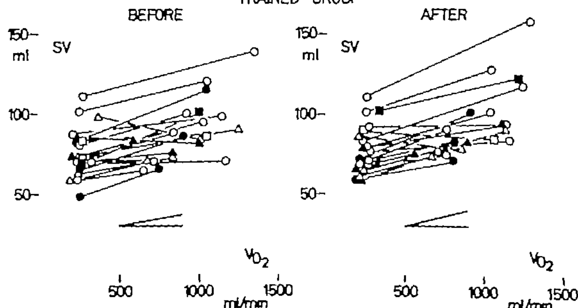


Fig 7. Arteriovenous ox[illegible] diff[illegible] work before and after the t[illegible] trained and [illegible]1 reference pat[illegible]



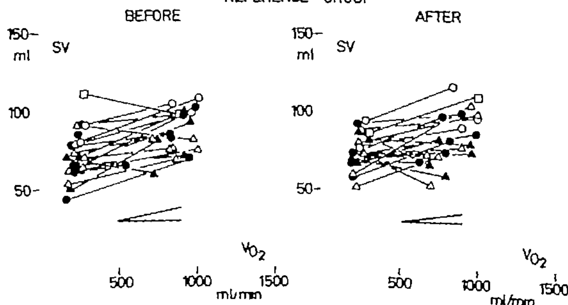


Fig. 8. Stroke volume (SV) related to oxygen uptake ($\dot{V} = \dot{V}O_2$) at rest and during supine work in 21 trained and 1 reference patients before and after the training or reference period. Symbols as in Figure 4. After training there was significant ($P<0.02$) increase of the mean slope of the rest-work line.

illustrates how AVD_{O_2} increased in all patients with increasing oxygen uptake. There were no significant changes between the mean slopes (rest-work) either before or after the training or reference period. Neither were there any significant differences between the trained and reference group.

The *cardiac output* showed no significant changes after the training or reference period, compared with

Fig 6 Cardiac output (Q) during work before and after the training or reference period in 21 trained and 21 reference patients Symbol as in Figure 4

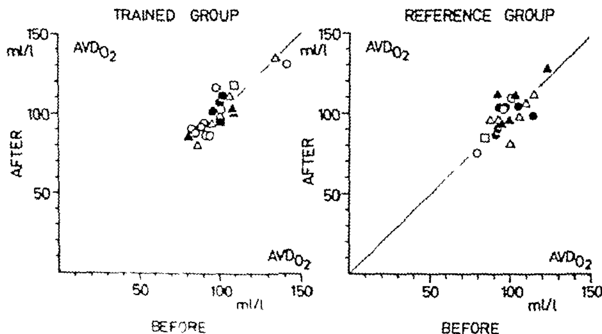


Fig 7 Arteriovenous ... work before and after the ... trained and 21 reference pa...

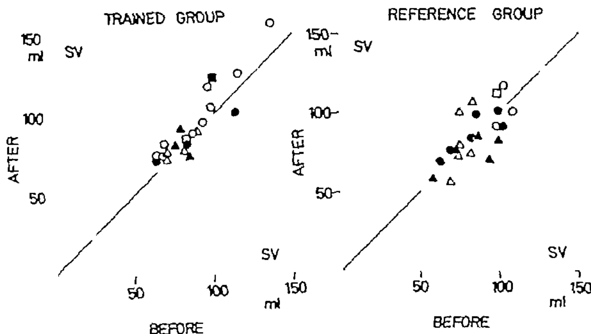


Fig. 10 Strok volume (SV) during ork before and after the training or reference period in 21 trained and 1 reference patients. Symbols as in Figure 4.

before The younger group had larger cardiac out puts than the older one in both groups, but there were no significant differences I *Figure 5* it is seen how the cardiac output increased with increasing oxygen uptake in all patients Th patients are compared with healthy men aged ₋3—41 years (40) and 61–83 years (79).

In *Figure 6* it is seen that the cardiac output of th younger patients remained unchanged after training also in the older patients and those with angina pectoris there was no clear tendency towards a lower cardiac output at the end of the training period The two older patients with an increased cardiac output after training (cases 18 and 30) were 58 and 59 years old, respectively The patient with angina pectoris had it very mildly and had a maximal heart rate of about 150 beats/min at the training sessions

The *cardiac index* was normal in both groups and no significant changes were observed after the training or reference period.

Figure 7 presents th *arteriovenous oxygen difference* before and after th training or reference period. It can be seen that there were no changes of AVD_{O_2} after th training period.

In the trained group th re was significant increase of the *stroke volume* (Table VII), which was more pronounced in the younger patients The largest increase was noted in the 9 younger patients *without* angina pectoris, from 93 to 103 ml ($P<0.01$) The reference group showed no significant changes of SV The younger patients of both groups had larger stroke volumes than the older ones, but these differences were not significant.

Figure 8 illustrates the relation between SV and $\dot{V}_{O_2}$ under conditions of est and exercise The mean slope of the rest work line was significantly increased after the training period ($P<0.05$). In the referenc group there was no significant change

Figure 9 presents th relation between SV and HR at rest and during exercise Here also th slope was significantly ncreased ($P<0.01$) after the training period. In th ref rence group there was no significant change A significant difference was found between the two main groups after the training or reference period ($P<0.05$).

In *Figure 10* it can be seen that the stroke volume incr ment of th trained group was due mainly to the younger patients, who initially showed a larger value than th others. The filled square symbol indicates a 48-year-old man (case 18) with questionable angina pectoris I the reference group the picture is less clear On multiple regression analysis, with the

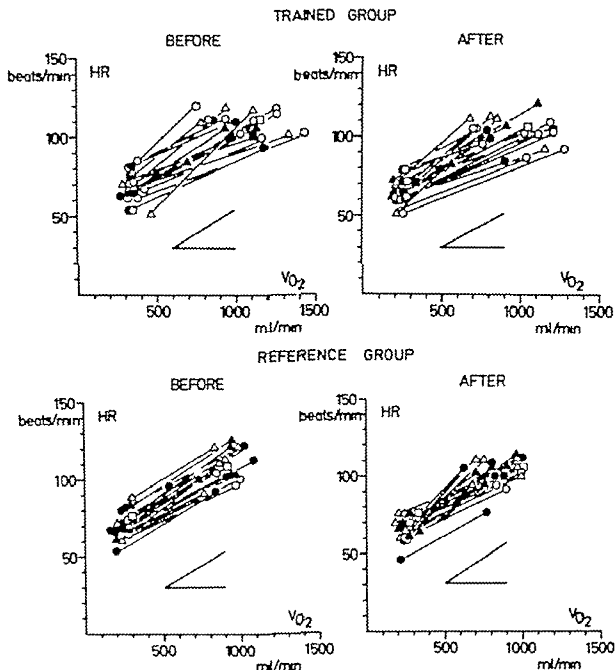


Fig. 11. Heart rate (HR) related to oxygen uptake (V_{O_2}) at rest and during work in the supine position before and after the training or reference period in 21 trained and 21 reference patients. Symbols as in Figure 4.
After training there was significant decrease (P<0.01) of the mean slope of the rest work line in the trained group. There is also significant difference (P 0.001) in slope between the tw groups after he training or reference period.

Table VII. *Haemodynamic flow data at work before and after the physical training or reference period (B).*

		Stroke volume (ml)		Heart rate (beats/min)		Pulmonary vasc. resist. (mm Hg/l/min)		Left ventricular minute work (kpm/min)		Left ventricular stroke work (g m)	
		Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP											
Total	Mean	88 P<0.02	94	107 P<0.001	100	1.3	1.1	17.5 P<0.01	15.6	163	155
	SEM	4.11	4.87	2.15	2.07	0.18	0.13	0.86	0.87	9.34	8.53
	Range	65-138	72 159	85 120	83-120	0.0-3.1	0.3-2.5	11.4-24.7	10.7 22.8	103-238	104-218
	N	21		22		18		17		17	
< 55 yrs	Mean	89 P<0.01	95	110 P<0.001	101	1.2	1.0	18.3 P<0.01	16.2	167	161
	SEM	5.47	6.59	2.35	2.11	0.20	0.10	1.09	0.90	11.5	10.4
	Range	65-138	74-159	88-120	86-110	0.4-3.1	0.4-1.6	13.1 24.7	12.5 21.2	111 238	120-218
	N	14		15		12		11		11	
> 55 yrs	Mean	87	90	103	99	1.4	1.1	16.1	14.6	155	144
	SEM	6.26	6.82	4.25	4.96	0.37	0.34	16.1	1.86	16.9	14.8
	Range	65-115	72 124	85-117	83-120	0.0-2.5	0.3-2.5	11.4-20.6	10.7 22.8	103-224	104-190
	N	7		7		4		6		6	
REFERENCE GROUP											
Total	Mean	85	85	107 P<0.05	103	1.0	1.1	16.5	16.2	156	159
	SEM	3.19	3.71	2.23	1.98	0.14	0.16	0.81	0.67	8.00	7.01
	Range	63-109	56-117	92 122	76-114	0.0-2.3	0.4-2.7	11.1 25.2	11.8 21.4	111 247	105-227
	N	21		21		18		18		18	
≤ 55 yrs	Mean	87	90	109 P<0.01	103	1.3	1.1	17.2	16.4	159	160
	SEM	4.33	6.28	3.00	2.43	0.17	0.15	1.10	1.05	13.4	11.6
	Range	75-109	56-117	96-122	91 112	0.5-2.3	0.4-1.5	13.9 25.2	11.8-21.4	124 247	105-227
	N	10		10		9		9		9	
> 55 yrs	Mean	83	80	104	102	0.7	1.1	15.7	15.9	152	159
	SEM	4.63	3.91	3.24	3.18	0.18	0.23	1.20	0.90	9.45	8.75
	Range	63-103	68-101	92 122	76-114	0.0-1.7	0.5-2.7	11.1 20.0	12.7 21.0	111 200	122-194
	N	11		11		9		9		9	

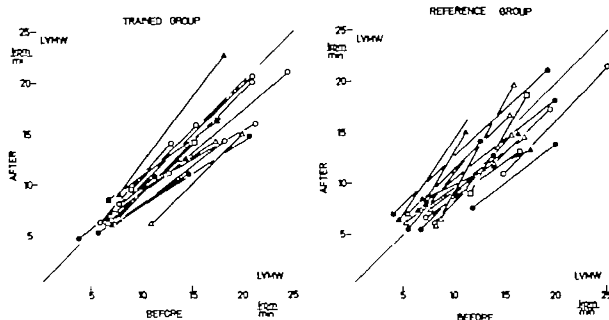


Fig 12. Left ventricular minute work (LVMW) at rest and during work before and after the training or reference period in 17 trained and 18 reference patients. Symbols as in Figure 4

change of the stroke volume during exercise after the reference or training period on the y axis and the initial value during exercise on the x axis, using age, residence and presence or absence of angina pectoris as independent variables, no significant change of the stroke volume was found after the training or reference period

The mean *heart rate* (Table VII) decreased slightly but significantly in both main groups. This was due to the younger patients because in the older patients the heart rate was essentially the same before and after the train ng or reference period *Figure 11* shows the relation between HR and V_{O_2} under conditions of rest and exercise In the trained group a significantly smaller slope (rest work) was noted after the training period (P<0.01) In the reference group there was no significant change There was a significant difference (P<0 001) between the main groups after the training or reference period.

The *pulmonary ascular esistance* was normal be fore and after the training or reference period, and no significant differences were found between the groups (Table VII)

Table VII also prese t data for th *left entricular minute work*. After training there was gnificant de crease (P<0 01), while th r feren group the final value was essentiall th sam th it al o e

The decrease of LVMW was achieved mainly be cause of the reduction of the mean arterial blood pressure after training In *Figure 12* it is seen that all trained patients except three had a lower value after the training. The patient with the largest increase (case 30) was 59 years old and his increase was due to an increment of the cardiac output from 9.3 to 11 3 l/min. In the reference group both increases and decreases were noted, but of the 7 patients with an increase all belonged to the older age group or had angina pectoris, except one patient (case 22).

Multiple regression analysis, with the change of LVMW during exercise after the training or refer ence period on the y axis and the initial value during exercise on the x axis, using age and the presence or absence of angina pectoris as independent variables, showed no significant change of LVMW after the training or reference period.

The *left entricular stroke ork* showed no significant changes or differences between either the main groups or the age groups. Lower values were observed in the older patients of both groups

Pressures

Table VIII present data for the *pulmonary arterial* nd *pulmonary cap llary enous pressures.* The PA

Table VIII. Haemodynamic pressure data (mm Hg) at work before and after the physical training or reference period. The final values for the diastolic pressure in the pulmonary artery differed significantly between the two younger groups (P<0.05)

		Pulmonary artery (PA)						PCV	
		Systolic		Mean		Diastolic		Mean	
		Before	After	Before	After	Before	After	Before	After
TRAINED GROUP									
Total	Mean	48	46	35	33	22	22	26	24
	SEM	2.49	3.05	2.25	2.26	1.43	1.54	2.25	2.02
	Range	27-70	23-80	15-58	14-58	10-32	8-38	5-45	7-45
	N	22		22		22		19	
≤55 yrs	Mean	47	46	33	34	21	22	25	24
	SEM	3.37	4.01	3.03	2.94	1.74	1.78	3.10	2.78
	Range	27-70	23-80	15-58	14-58	10-30	8-35	5-45	7-45
	N	15		15		15		13	
>55 yrs	Mean	52	47	38	33	25	22	28	24
	SEM	3.87	4.66	2.69	3.62	2.37	3.11	2.44	2.48
	Range	45-67	35-70	27-47	24-50	16-32	16-38	20-37	16-32
	N	7		7		7		6	
REFERENCE GROUP									
Total	Mean	43	44	31	31	20	20	21	21
	SEM	3.06	2.51	2.67	2.07	1.94	1.61	2.71	2.12
	Range	18-66	30-70	12-50	18-48	7-35	10-34	8-46	9-45
	N	19		19		19		19	
≤55 yrs	Mean	40	39	28	26	19	16	17	17
	SEM	3.48	2.47	3.20	1.87	2.61	1.26	2.16	1.85
	Range	23-53	31-53	14-45	18-36	9-35	10-22	8-28	9-28
	N	9		9		9		10	
>55 yrs	Mean	46	48	34	35	21	23	25	25
	SEM	4.88	3.83	4.12	2.97	2.94	2.56	4.93	3.51
	Range	18-66	30-70	12-50	22-48	7-34	10-34	10-46	15-45
	N	10		10		10		9	

pressures were abnormally increased in comparison with healthy old men 61—81 years (29). A tendency to higher pressures in the older than in the younger patients of the two groups was noted. There were no significant changes before or after the training or reference period.

The mean PCV pressure was also abnormally increased in comparison with that in healthy old men (29), with a tendency to higher pressures in the older age groups. No significant changes in the PCV pressure were noted after the training or reference period.

The *systemic arterial* pressures are presented in *Table IX*. On comparison with healthy old men (29) the pressures lay at the upper normal limit at a corresponding work load. In the reference group there were no significant changes but in the trained group there was a significant decrease of both the *systolic, mean and diastolic* pressures. The pressures of case 25 in the reference group were excluded from the calculations because they probably were not correct (at the first examination his *systolic pressure* was 115 mm Hg and at the second examination it was 250 mm Hg).

Table IX. *Pressures (mm Hg) in the systemic artery at work before and after the physical training or reference period. After the training or reference period there were significant differences between the main groups both in the systolic (P<0.05) mean (P<0.01) and diastolic (P<0.05) pressures. The final values for the older patients in each main group also differed significantly (P<0.05), except those for systolic pressure.*

		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After
TRAINED GROUP							
Total	Mean	183 P<0.05	172	124 P<0.01	115	88 P<0.05	82
	SEM	6.15	5.36	3.83	3.67	2.46	3.16
	Range	150-240	145-220	105-165	90-140	70-110	60-110
	N	18		18		18	
<55 yrs	Mean	183	173	125	115	90	88
	SEM	8.13	6.20	5.38	3.96	3.39	4.10
	Range	150-240	150-210	105-165	90-135	70-110	60-110
	N	12		12		12	
>55 yrs	Mean	182	171	123 P<0.05	108	86	78
	SEM	9.75	11.8	4.62	7.74	3.01	6.02
	Range	150-210	145-220	110-135	90-140	75-90	70-100
	N	6		6		6	
REFERENCE GROUP							
Total	Mean	183	188	124	128	91	92
	SEM	6.62	7.12	4.00	4.35	2.88	3.04
	Range	145-255	150-270	100-165	110-180	75-115	80-120
	N	19		18		19	
<55 yrs	Mean	173	174	126	121	93	89
	SEM	10.7	7.53	5.42	3.05	3.63	3.41
	Range	145-255	150-225	110-165	110-135	80-115	80-105
	N	9		9		9	
>55 yrs	Mean	192	201	123	136	89	96
	SEM	7.46	10.5	6.18	7.48	4.46	4.85
	Range	160-250	160-270	100-160	110-180	75-110	80-120
	N	10		9		10	

In *Figure 13* it is seen that in all trained patients except three there was a reduction of the mean arterial pres ure after the training period. In at least one of these (case 13 with initial alue 110 and final alue 130 mm Hg) the training intensity w not ery high because of angina pectoris and a tendency to entricu lar ectopic beats during exercise. I the reference patient both increases and decreases ere recorded the three cases with the greatest increase (ases 1, 13 and 29) were all more than 60 years old and had angina pectoris.

Multiple regression analysis, with the difference between the mean arterial blood pressure during exercise after and before the training or reference period on the y-axis and the blood pressure during exercise before these periods on the x axis, and with ge and the presence or absence of ang na pectoris as independent ariables, showed a *significantly lower* blood pressure in the trained group as compared with th reference group (P 0.01).

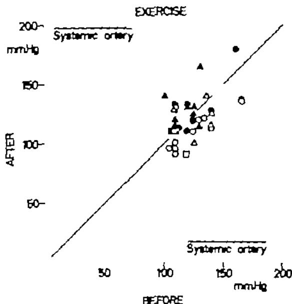


Fig 13 Systemic arterial (SA) pressure during work before and after the training or reference period in 18 trained and 18 reference patients.
Open symbols represent patients of the trained group, and *filled* symbols patients of the reference group. *Triangles* represent patients with angina pectoris and *squares* patients with questionable angina pectoris. *Circles* represent patients without angina pectoris.

Table IX. *Pressures (mm Hg) in the systemic artery at work before and after the physical training or reference period. After the training or reference period there were significant differences between the main groups both in the systolic (P<0.05), mean (P<0.01), and diastolic (P<0.05) pressures. The final values for the older patients in each main group also differed significantly (P<0.05), except those for systolic pressure.*

		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After
TRAINED GROUP							
Total	Mean	183 P<0.05	172	124 P<0.01	113	88 P<0.05	82
	SEM	6.15	5.36	3.83	3.67	2.46	3.16
	Range	150-240	145-220	105 165	90-140	70-110	60-110
	N	18		18		18	
<55 yrs	Mean	183	173	125	115	90	88
	SEM	8.13	6.20	5.38	3.96	3.39	4 10
	Range	150-240	150-210	105-165	90-135	70-110	60-110
	N	12		12		12	
>55 yrs	Mean	182	171	123 P<0.05	108	86	78
	SEM	9 75	11.8	4.62	7 74	3.01	6.02
	Range	150-210	145-220	110-135	90-140	75-90	70-100
	N	6		6		6	
REFERENCE GROUP							
Total	Mean	183	188	124	128	91	92
	SEM	6.62	7.12	4.00	4.35	2.88	3.04
	Range	145-255	150-270	100-165	110-180	75 115	80-120
	N	19		18		19	
<55 yrs	Mean	173	174	126	121	93	89
	SEM	10 7	7.53	5 42	3.05	3.63	3 41
	Range	145 255	150-225	110-165	110-135	80-115	80-105
	N	9		9		9	
>55 yrs	Mean	192	201	123	136	89	96
	SEM	7 46	10.5	6 18	7.48	4 46	4.85
	Range	160-250	160-[illegible]70	100-160	110-180	75 110	80-120
	N	10		9		10	

In *Figure 13* it is seen that in all trained patients except three there was a reduction of the mean arterial pressure after the training period. In at least one of these (case 13 with initial value 110 and final value 130 mm Hg) the training intensity was not very high because of angina pectoris and tendency to ventricular ectopic beats during exercise. In the reference patients both increases and decreases were recorded; the three cases with the greatest increase (cases 1, 13 and [illegible]9) were all more than 60 years old and two had angina pectoris.

Multiple regression analysis, with the difference between the mean arterial blood pressure during exercise after and before the training or reference period on the y axis and the blood pressure during exercise before these periods on the x axis, and with age and the presence or absence of angina pectoris as independent variables, showed a significantly lower blood pressure in the trained group as compared with the reference group (P<0.01).

support the view that the increase in work capacity and reduction of cardiac work after physical training in patients with coronary artery disease are due to effects on the peripheral circulation.

Frick (25 26), on the other hand, observed no significant decrease of the cardiac output or increase of the arteriovenous oxygen difference during submaximal work after training of a group of six patients with earlier myocardial infarction, three of whom had angina pectoris (mean age 48 years) The increase of the stroke volume that he observed has been ascribed to improved contractility and muscular hypertrophy (24).

A reason for the varying results of the physical training can be differences in the *examination procedur* as some authors have examined th patients in the sitting posture (16 70) and others with the patient supine (26 27) The supine posture gives a more hyperkinetic state with optimal conditions for filling of the heart chambers, greater venous return a larger cardiac output, a higher blood pressure, a lower heart rate and a greater strok volume with this posture a strok volume increase after physical training is probably more easily obtained (39).

It seems that both *age* and the question of whether or not *angina pectoris* is present are of importance for the effect of the training In the present series there was an increase of th stroke volum only in the younger age group of the trained patients This stroke volume increase during submaximal exercise after training was still more pronounced in the younger patients without angina pectoris Whether this fact only reflects the lower training intensity in the older patients and those with angina pectoris or whether there are other differences also cannot be stated on the basis of the present results In cases with previous infarction and old age or angina pectoris more sever coronary heart disease can be presumed.

Perhaps it may be assumed that the old infarction patient, especially if he has had a large infarct, has little possibility of increasing his stroke volume during exercise after training because of the damaged myocardium In that case there will possibly be a compensatory increase of the peripheral circulatory effects after a period of physical training. The divergent results in the above mentioned studies (17 77 70) on small series of patients could then be explained by inhomogeneity of the series, i e larger infarcts in th series with mainly peripheral effects (17 71), and younger patients with no angina pectoris in the series with mainly central circulatory effects (27). All patients in the series with mainly peripheral effects also had angina of effort. In the study with mainly central effects only half (three of six) had angina pectoris. Th patients were also slightly younger in this latter study

CONCLUSIONS

During the approximately 1000 training sessions no arrhythmias or other serious complications occurred. The risk involved with this form of treatment can thus be said to be very small. The interest in and the rate of adherence to the treatment was very high among the patients, and only one of 34 discontinued the training for lack of motivation. Of the six patients whose training was terminated because of reinfarction, other disease, increasing severity of angina pectoris or breathlessness, five were 60 years old or older. This indicates that the older patient has smaller reserves than the younger and therefore more easily suffers over-exertion. The haemodynamic effect of the training was also less pronounced in the older group of patients (more than 55 years). No significant reduction of the work of the heart was observed in this group nor any increase of the stroke volume during exercise. On the other hand the mean arterial blood pressure during exercise was significantly lower after the training. In the younger group of patients (less than or equal to 55 years) there was a reduction of the heart rate, an increase of the stroke volume, a decrease of the mean arterial blood pressure and LVMW during exercise after the training period. The abnormally high pressures in the pulmonary circulation during work were unchanged after the training and reference period.

The objective improvement that takes place in the infarct patient after a period of physical training can be caused both by effects on the central and on the peripheral circulation. It is possible that the central effect is not so easily achieved in older patients, patients with angina pectoris or those with large infarcts. These patients therefore conceivably have a compensatorily larger effect on the peripheral circulation.

REFERENCES

1 Barry A.J. Daly J.W. Pruett, E.D.R. Stemmetz, J.R., Birkhead, N.C. & Rodahl, K., Effects of physical training in patients who have had myocardial infarction. Amer. J. Cardiol. 17 1 1966.

2 Barry A.J. Daly J.W. Pruett, E.D.R. Stemmetz, J.R., Page, H.F. Birkhead, N.C. & Rodahl, K. The effects of physical conditioning on older individuals. I Work capacity circulatory-respiratory function and work electrocardiogram J. Geront. 1 182, 1966

3 Benestad, A.M. Trainability of old men. Acta med. scand. 178 321 1965

4 Bergman, H. & Varnauskas, E. Placebo effect in physical training of coronary heart disease patients. I Physical Fitness and Coronary Heart Disease. Munksgaard, Copenhagen, 1971

5. Bergström, K. Bjernulf A. & Erikson, U. Work capacity heart and blood volume before and after physical training in male patients after myocardial infarction. Submitted to Scand. J. Rehab. Med

6 Bevegård, S., Holmgren, A. & Jonsson, B. Circulatory studies in well trained athletes at rest and during heavy exercise, with special reference to stroke volume and the influence of body position. Acta physiol. scand 57 26 1963

7 Bjernulf, A. & Boberg, J. Metabolic aspects of physical training after myocardial infarction. Submitted to Acta med. scand.

8 Bjernulf, A. & Johannesson, K. Psychological aspects of physical training after myocardial infarction. To be published.

9 Björk, L. Cullhed, I. & Bochbolz, B. Left ventricular function in ischemic heart disease. Acta med. scand. 190 223 1971

10 Bonica, J.J. Berges, P.U. & Morikawa, K. Circulatory effects of peridural block. Anesthesiology 33 619 1970

11 Bruce R.A. & Hornsten, T.R. Myocardial infarction after normal responses to maximal exercise. Circulation 38 552, 1968

12 Bruce R.A. & Hornsten, T.R. Exercise stress testing in evaluation of patients with ischemic heart disease. Progr. cardiovasc. Dis. 11 371 1969

13 Brunner D. A... exercise for coronary patients. Rehab. Res. 9 29 1965

14 Bruun, O.A. Rehabilitating coronary patients through exercise. Postgrad. Med. 44 131 1968

15 C... twell J.D. & Fletcher G.F. Cardiac complications while jogging. J. Amer. med. Ass. 10 130 1969

16 Clausen J.P. Larsen O.A. & Trap-Jensen J. Physical training in the management of coronary artery disease. Circulation 40 143 1969

17 Clausen, J.P. & Trap-Jensen, J. Effect of training on the distribution of cardiac output in patients with coronary artery disease. Circulation 42 611 1970

18. Du Bois, D. & Du Bois, E.F. A formula to estimate the approximate surface area if height and weight be known. Arch. Intern. Med. 17 863, 1916

19 Eilers, R.J. Notification of final adoption of an international method and standard solution for hemoglobinometry specifications for preparation of standard solution. Blood 29 285, 1967

20 Ekblom, B. Åstrand, P.O. Saltin, B. Stenberg, J. & Wallström, B. Effect of training on circulatory response to exercise. J. Appl. Physiol. 24 518, 1968

21 Enghoff, H. Apparat zur Gasanalyse. Acta med. scand. Suppl. 170 307 1946

22 Enghoff, E. & Enghoff, H. Zur Technik der Gaswechselbestimmungen. Kungl. Svenska Vetenskapsakademiens handlingar 4 1 1953

23 Forrester J.S. Kemp H.G. & Gorlin, R. Distinctive characterization of the patient with myocardial infarction and no angina pectoris. Clin. Res. 16 229 1968

24 Frick, M.H. The response of heart volume and ventricular functions to physical training in coronary heart disease. Mal. cardiovasc. 10 331 1969

25 Frick, M.H. The effect of physical training in manifest ischemic heart disease. Circulation 40 433, 1969

26 Frick, M.H. Effects of physical training in coronary heart disease. Verh. dtsch. Ges. Kreisl. Forsch. 37 94 1971

27 Frick, M.H. & Katila, M. Hemodynamic consequences of physical training after myocardial infarction. Circulation 37 192, 1968

28 Gottheiner V. Long-range strenuous sports training for cardiac reconditioning and rehabilitation. Amer. J. Cardiol. 22 426, 1968

29 Granath, A. Jonsson B. & Strandell T. Circulation in healthy old men studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta med. scand. 176 425 1964

30 Granath, A. & Strandell, T. Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. Acta med. scand. 176 447 1964

31 Hanson, J.S. Tabakin, B.S. Levy, A.M. & Nedde, W. Long-term physical training and cardiovascular dynamics in middle-aged men. Circulation 38 783, 1968

32 Harris, J.A. & Benedict, F.G. A biometric study of basal metabolism in man. Carnegie Inst. Wash. Publ. No. 79 1919

33 Hartley L.H. Grimby G. Kilbom, A. Nilsson, N.J. A... and I. Bjure J. Ekblom, B. & Saltin B. Physical training in sedentary middle-aged and older men. III Cardiac output and gas exchange at submaximal and

maximal exercise Scand. J. Clin. Lab. Invest. 24 335 1969
34 Harvey R.M. Smith, W.M. Parker J.O. & Ferrer I. The response of the abnormal heart to exercise Circulation 26 341 1962.
35 Hellerstein E.M. Four-year experience with a graded exercise program for postcoronary patients. Appl Ther 11 386, 1969
36 Hellerstein, H.K. Hirsch, E.Z., Cumler W.A.L., Polster S. & Zucker N. Reconditioning of the coronary patient Preliminary report / Coronary Heart Disease edited by W. Likoff & J.H. Moyer New York, Grune & Stratton, Inc 1963 p 448
37 Hoel, P.G. Elementary statistics, 2nd ed. John Wiley and Sons, Inc New York 1967
38 Holloszy J.O. Biochemical adaptations in muscle Effect of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol. Chem 42 278 1967
39 Holmgren, A. Personal communication
40 Holmgren, A. Jonsson, B. & Sjöstrand, T. Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke volume at different work intensities. Acta physiol scand 49 343 1960.
41 Holmgren, A. & Mattsson, K.-H. A new ergometer with constant work load at varying pedalling rate Scand J Clin Lab Invest 6 137 1954
42 Holmgren, A. Mossfeldt F. Sjöstrand, T. & Strom G. Effect of training on work capacity total hemoglobin, blood volume heart volume and pulse rate in recumbent and upright positions Acta physiol scand 50 7 1960
43 Holmgren A. & Pernow B. Spectrophotometric measurement of oxygen saturation of blood the determination of cardiac output. A comparison with the Van Slyke method Scand J Clin Lab Invest 11 143 1959
44 Kaijser L. B. Michaelsson M. & Strom, G. A comparison between glass and lucite cuvettes in spectrophotometric determination of haemoglobin oxygen saturation Scand J Clin Lab Invest 57 1968
45 Kellerman, J.J. Modan B. Levy M. Feldman, S. & Karns I. Return to work after myocardial infarction Geriatrics 3 151 1968
46 Kilbom, A. Hartley L.H. Saltin B. Bjure J. Grimby G. & Astrand I. Physical training in sedentary middle aged and older men. I. Medical evaluation. Scand J Clin Lab Invest 4 315 1969
4 Malmborg R.O. A clinical and hemodynamic analysis of factors limiting the cardiac performance in patients with coronary heart disease Acta med scand Suppl 426 196
4 Malmcrona, R. C. ... & ... Haemodynamic data during rest and ... in patients who have ... been ... myocardial infarction Acta med scand ...
4 Malmcrona R. & Varnauskas E. Haemodynamics during rest and exercise at the end of convalescence from myocardial infarction Acta med scand ... 1964
Mitchell J.H. ... Billings, T.I. Exercise to prevent coronary heart disease Amer J Med. 46 12, 1969
51 M H rry P.L. Fisch C., Jordan, F.J.W. & Corya, B.R. Cardiac arrhythmias observed during maximal treadmill exercise testing in clinically normal men. Amer J Cardiol. 29 331 1972.
52. Mitchell, J.H. Hefner L.L. & Monroe, R.G. Performance of the left ventricle Amer J Med 53 481 1972.
53 Müller O. & Rorvik K. Haemodynamic consequences of coronary heart disease Brit Heart J 20 302, 1958
54 Naughton, J. Bruhn, J.G. & Lategola, M.T. Effects of physical training on physiologic and behavioral characteristics of cardiac patients. Arch Phys. Med 49 131 1968
55 Naughton J. Shanbour K. Armstrong, R., McCoy J. & Lategola, M.T. Cardiovascular responses to exercise following myocardial infarction Arch Intern Med. 117 541 1966
56 Sy... E.R. Exercise in the treatment of coronary artery disease J Roy Coll Physcns Lond 6 69 1971
57 Pyfer H.R. & Doane B.L. Cardiac arrest during exercise training J Amer med Ass 210 101 1969
58 Rahimtoola, S.H. DiGilio M.M. Sinno, M.Z., Loeb H.S. Rosen, K.M. & Gunnar R.M. Cardiac performance three to eight weeks after acute myocardial infarction Arch Intern. Med 128 220, 1971
59 Rechnitzer P.A. Pickard, H.A. Paivio A.U. Yuhasz M.S. & Cunningham, D. Long-term follow-up study of survival and recurrence rates following myocardial infarction in exercising and control subjects. Circulation 45 853 197
60 Rose G.A. The diagnosis of ischaemic heart pain and intermittent claudication field surveys Bull Wld Hlth Org 27 645 196
61 Saltin, B. Blomqvist G. Mitchell J.H. Johnson, R.L. Wildenthal, K. & Chapman, C.B. Response to exercise after bed rest and after training Circulation Suppl VII 1968
6 Sanne H. Grimby G. & Wilhelmsen, L. Physical training during convalescence after myocardial infarction / Physical Fitness and Coronary Heart Disease M. Larsen ... Copenhagen 1971
63 Seldinger S.I. Catheter replacement of the needle percutaneous arteriography Acta radiol 39 368 1953
64 Sjöstrand, T. Bestämning av den fysiska arbetsförmåga såsom klinisk undersökningsmetod Svenska Läk Tidn 47 347 1950
65 Sonnenblick E.H. Ross, J. & Braunwald E. Oxygen consumption of the heart Amer J Cardiol 378 1968
66 Stead, E.A. Warren J.V. Merrill, A.J. & Brannon, E.S. The cardiac output in male subjects as measured by the technique of right atrial catheterization Normal values with observation of the effect of anxiety and tilting J Clin Invest 4 376, 194
Stephens ... T. ... absorbency of ... methaemoglobin Scand J Clin Lab Invest ... 1967
... J.S. & Zohman L.R. ... coronary patient Postgrad Med 47 139 1970
W... d H Determination of the physical working

capacity Acta med scand Suppl 15 1948
70 Varnauskas, E., Bergman, H Houk, P & Bjöntorp P Haemodynamic effects of physical training in coronary patient Lancet 2, 1966.
71 Varnauskas, E., Björntorp P Fahlén, M Prerovsky I & Stenberg, J Effects of physical training on exercise blood flow and enzymatic activity in skeletal muscle Cardiovasc Res 4 418 1970
72. Wiener L., Dwyer E.M & Cox, J W Left ventricular hemodynamics in exercise-induced angina pectoris Circulation 38 40, 1968.
73 Wanne B Circulatory adaptation early after pulmonary surgery Acta Univ upsal. Abstracts of Uppsala Dissertations in Medicine 83, 1970

maximal exercise. Scand. J. Clin. Lab. Invest. 4:335 1969

34. Harvey, R.M., Smith, W.M., Parker, J.O. & Ferrer, I. The response of th[e] abnormal heart to exercise. Circulation 26:341 196_

35. Hellerstein, E.M. Four-year experience [wi]th a graded exercise program for postcoronary patients. Appl. Ther. 11:386 1969

36. Hellerstein, H.K., Hirsch, E.Z., Cumler, W.A.L., Polster, S. & Zucker, N. Reconditioning of the coronary patient. Preliminary report. I. Coronary Heart Disease, edited by W. Likoff & J.H. Moyer. New York, Grune & Stratton, Inc. 1963 p. 448

37. Hoel, P.G. Elementary statistics, 2nd ed. John Wiley and Sons, Inc. New York 1967

38. Holloszy, J.O. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. Biol. Chem. 4_ _278 1967

39. Holmgren, A. Personal communication

40. Holmgren, A., Jonsson, B. & Sjöstrand, T. Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke volume at different work intensities. Acta physiol. scand. 49:343 1960

41. Holmgren, A. & Mattsson, K.-H. A new ergometer with constant work load at varying pedalling rate. Scand. J. Clin. Lab. Invest. 6:137 1954

42. Holmgren, A., Mossfeldt, F., Sjöstrand, T. & Ström, G. Effect of training on work capacity, total hemoglobin, blood volume, heart volume and pulse rate in recumbent and upright positions. Acta physiol. scand. 50:72, 1960

43. Holmgren, A. & Pernow, B. Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison [wi]th the V[an] Slyke method. Scand. J. Clin. Lab. Invest. 11:143 1959

44. Karendal, B., Michaelsson, M. & Ström, G. A comparison between glass and lucite cuvettes in spectrophotometric determination of haemoglobin oxygen saturation. Scand. J. Clin. Lab. Invest. _ 57 1962

45. Kellerman, J.J., Modan, B., Levy, M., Feldman, S. & Kariv, I. Return [to w]ork after myocardial infarction. Geriatrics _3 1 1 1968

46. Kilbom, A., Hartley, L.H., Saltin, B., Bjure, J., Grimby, G. & Astrand, I. Physical training in sedentary middle aged and older men. I. Medical evaluation. Scand. J. Clin. Lab. Invest. 4:31_ 1969

47. Malmborg, R.O. A clinical and hemodynamic analysis of factors limiting [t]h[e c]ardiac performance in patients [wit]h coronary heart disease. Acta med. scand. Suppl. 426 1965

48. Malmcrona, R., Cramer, G. & Varnauskas, E. Hemodynamic data during rest and exercise for patients who have or have not been able to return their occupation after myocardial infarction. Acta med. scand. 1_4 1965

49. Malmcrona, R. & Varnauskas, E. Haemodynamics during rest and exercise at the end of convalescence from myocardial infarction. Acta med. scand. 1_ 19 1964

50. Man_ G.V., Garrett, H.L., Farh_, A., M_r_ H. & Billings, T.F. Exercise to prevent coronary heart disease. Amer. J. Med. 46:1_ 1969

51. McHenry, P.L., Fisch, C., Jordan, F.J.W. & Corya, B.R. Cardiac arrhythmias observed during maximal treadmill exercise testing in clinically normal men. Amer. J. Cardiol. 29:331 1972

52. Mitchell, J.H., Hefner, L.L. & Monroe, R.G. Performance of the left ventricle. Amer. J. Med. 3:481, 197_

53. Muller, O. & Rorvik, K. Haemodynamic consequences of coronary heart disease. Brit. Heart J. 20:90_ 1958

54. Naughton, J., Bruhn, J.G. & Lategola, M.T. Effects of physical training on physiologic and behavioral characteristics of cardiac patients. Arch. Phys. Med. 49:131 1968

55. Naughton, J., Sharhout, K., Armstrong, R., McCoy, J. & Lategola, M.T. Cardiovascular responses to exercise following myocardial infarction. Arch. Intern. Med. 11_ 541 1966

56. Nye, E.R. Exercise in the treatment of coronary artery disease. J. Roy. Coll. Phycns Lond. 6:69 1971

57. Pyfer, H.R. & Doane, B.L. Cardiac arrest during exercise training. J. Amer. med. Ass. 10:101, 1969

58. Rahimtoola, S.H., DiGilio, M.M., Sinno, M.Z., Loeb, H.S., Rosen, K.M. & Gunnar, R.M. Cardiac performance three to eight weeks after acute myocardial infarction. Arch. Intern. Med. 128:220, 1971

59. Rechnitzer, P.A., Pickard, H.A., Paivio, A.U., Yuhasz, M.S. & Cunningham, D. Long-term follow-up study of survival and recurrence rates following myocardial infarction [in] exercising and control subjects. Circulation 45:853 1972

60. Rose, G.A. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull. Wld Hlth Org. 27:645 196_

61. Saltin, B., Blomqvist, G., Mitchell, J.H., Johnson, R.L., Wildenthal, K. & Chapman, C.B. Response to exercise after bed rest and after training. Circulation Suppl. VII 1968

62. Sanne, H., Grimby, G. & Wilhelmsen, L. Physical training during convalescence after myocardial infarction. I. Physical Fitness and Coronary Heart Disease. Munksgaard, Copenhagen, 1971

63. Seldinger, S.J. Catheter replacement of the needle in percutaneous arteriography. Acta radiol. 39:368 1953

64. Sjöstrand, T. Bestämning av den fysiska arbetsförmågan såsom klinisk undersökningsmetod. Svenska Läk. Tidn. 47:349 1950

65. Sonnenblick, E.H., Ross, J. & Braunwald, E. Oxygen consumption of the heart. Amer. J. Cardiol. _ 328 1968

66. Stead, E.A., Warren, J.V., Merrill, A.J. & Brannon, E.S. The cardiac output in male subjects as measured by the technique of right atrial catheterization. Normal values [wit]h observations of the effects of anxiety and tilting. J. Clin. Invest. 4:326 1945

67. Sigbrand, T. Molar absorbancy of cyanmethaemoglobin. Scand. J. Clin. Lab. Invest. 20:25_ 1967

68. Tobis, J.S. & Zohman, L.R. Rehabilitating the coronary patient. Postgrad. Med. 4_ 139 1970

69. Wahlund, H. Determination of the physical working

Table XIX. *Left ventricular minute work (LVMW) (kpm/min) at rest and during exercise before and after the training or reference period.*

TRAINED GROUP

	Rest		Exercise	
Case	Before	After	Before	After
1	12.7	11.1	24.7	21.2
3	7.6	7.4	–	–
4	7.2	5.3	14.0	12.5
7	8.9	9.5	15.5	15.9
10	5.8	5.2	14.7	11.0
15	6.5	6.4	21.0	20.7
18	6.8	8.4	17.5	16.3
19	7.8	6.7	21.3	20.0
21	6.0	6.2	13.1	14.0
22	6.7	6.3	15.2	14.1
25	7.0	6.1	20.6	14.7
26	3.9	4.7	11.4	10.7
29	6.6	5.6	14.4	12.4
30	7.8	8.0	18.1	22.8
31	11.2	5.4	20.0	15.3
32	7.8	8.2	21.2	15.9
33	7.4	5.9	18.2	14.1
34	7.6	7.5	17.3	14.4
Mean	7.5	6.9	17.5	15.6
SEM	0.46	0.39	0.86	0.87
Range	3.9-12.7	4.7-11.1	11.4-24.7	10.7-22.8
N	18		17	

REFERENCE GROUP

	Rest		Exercise	
Case	Before	After	Before	After
1	6.3	7.3	11.1	15.0
2	5.6	5.5	12.5	14.0
3	7.6	9.0	–	–
4	7.2	6.8	16.4	13.2
5	7.1	7.9	16.2	14.7
7	14.7	10.7	25.2	21.4
10	8.5	6.3	15.6	14.8
13	4.4	6.5	11.5	16.5
14	7.5	7.5	16.7	14.5
17	6.8	8.5	17.3	13.1
19	5.3	7.3	15.4	16.5
20	8.2	5.3	15.7	19.7
21	11.8	7.7	20.0	17.8
22	11.5	9.0	17.0	18.7
23	8.2	6.5	19.3	17.3
24	5.3	6.6	13.9	11.8
27	7.4	8.3	19.9	18.2
28	6.7	5.5	13.8	12.7
29	4.0	7.1	19.4	21.0
Mean	7.6	7.3	16.5	16.2
SEM	0.60	0.31	0.81	0.67
Range	4.0-14.7	5.3-10.7	11.1-25.2	11.8-21.4
N	19		18	

Table XX. *Left ventricular stroke work (LVSW) (g m) at rest and during exercise before and after the training or reference period.*

TRAINED GROUP

	Rest		Exercise	
Case	Before	After	Before	After
1	177	154	238	206
3	104	108	–	–
4	101	88	133	120
7	105	123	139	169
10	81	78	135	114
15	107	110	184	204
18	95	116	175	161
19	135	135	209	218
21	89	99	111	132
22	98	103	136	133
25	130	102	224	176
26	62	75	103	104
29	93	81	137	118
30	121	118	156	190
31	135	115	167	140
32	124	144	192	159
33	137	118	185	165
34	91	97	145	129
Mean	110	109	163	155
SEM	6.25	5.13	9.34	8.53
Range	81-177	78-154	103-238	104-218
N	18		17	

REFERENCE GROUP

	Rest		Exercise	
Case	Before	After	Before	After
1	101	111	111	140
2	105	120	136	185
3	91	126	–	–
4	114	111	171	144
5	104	120	158	138
7	201	178	247	227
10	97	84	130	135
13	67	108	124	171
14	91	98	137	135
17	94	125	169	224
19	76	117	140	166
20	126	78	132	177
21	152	127	200	182
22	152	118	157	176
23	127	114	196	171
24	82	104	124	105
27	112	133	178	165
28	84	82	133	132
29	60	125	159	194
Mean	107	115	156	159
SEM	7.74	5.05	8.00	7.01
Range	60-201	78-178	111-247	105-227
N	19		18	

Table XXI. *Pressures in the right atrium (RA) and right ventricle (RV) (mm Hg) at rest before and after the training or reference period.*

TRAINED GROUP

Case	RA Mean Before	RA Mean After	RV Systolic Before	RV Systolic After	RV End-diastolic Before	RV End-diastolic After
1	3	4	22	16	5	0
2	3	5	25	22	4	6
3	3	5	25	25	4	3
4	3	1	23	23	1	2
7	−2	0	22	22	−2	−2
8	4	6	25	25	7	7
10	4	2	20	23	4	1
13	2	2	25	23	3	4
15	2	2	20	28	3	6
18	7	7	24	16	6	3
19	3	3	19	25	3	6
21	3	3	26	30	0	4
22	2	2	24	22	0	0
23	5	5	22	27	3	5
25	5	5	25	20	3	3
26	3	3	18	19	3	2
27	4	4	27	29	4	3
29	4	4	21	26	4	6
30	4	4	36	26	5	5
31	6	6	26	24	6	3
32	1	1	24	24	3	3
33	4	4	20	22	5	4
34	1	1	20	24	1	3
Mean	3	3	23	24	3	3
SEM	0.38	0.38	0.78	0.74	0.45	0.46
Range	-2-7	0-7	18-36	16-30	−2-7	−2-7
N	23		23		23	

REFERENCE GROUP

Case	RA Mean Before	RA Mean After	RV Systolic Before	RV Systolic After	RV End-diastolic Before	RV End-diastolic After
1	2	1	36	29	6	3
2	2	1	18	25	1	3
3	4	2	25	23	6	4
4	3	3	23	22	4	2
5	2	3	–	–	–	–
7	1	3	19	23	0	3
8	3	3	16	26	1	3
10	3	6	21	24	0	5
13	−1	4	15	20	−2	2
14	2	4	25	27	2	5
17	2	2	20	25	3	3
19	4	3	16	19	–	–
20	4	2	28	17	5	3
21	3	0	21	20	3	1
22	−1	0	20	24	3	3
23	3	1	28	24	5	2
24	2	2	20	20	3	4
25	4	1	26	34	4	5
27	4	2	23	24	5	3
28	0	2	20	20	2	2
29	3	4	24	28	2	3
Mean	2	2	22	24	3	3
SEM	0.33	0.40	1.11	0.89	0.50	0.25
Range	1-4	0-6	16-36	17-34	−2-6	2-6
N	21		20		19	

Table XXII. *Pressures in the pulmonary artery (PA) (mm Hg) at rest before and after the training or reference period.*

TRAINED GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Before	Diastolic After
1	19	15	12	10	8	6
2	21	16	14	11	10	8
3	22	23	14	12	7	6
4	26	20	17	13	11	8
7	15	16	8	9	2	6
8	16	19	11	15	7	11
10	20	17	12	10	6	5
13	23	21	14	13	7	5
15	17	18	10	11	6	5
18	28	18	19	11	14	7
19	16	24	10	16	7	8
21	25	27	18	18	12	12
22	24	23	14	12	7	6
23	17	16	12	12	8	8
25	23	20	13	11	6	6
26	16	18	10	10	6	6
27	20	22	12	11	7	5
29	20	26	12	16	6	10
30	25	28	16	15	8	10
31	24	20	14	13	9	9
32	26	26	13	20	6	14
33	22	22	14	14	8	8
34	29	25	13	14	7	9
Mean	22	21	13	13	8	8
SEM	0.85	0.81	0.55	0.58	0.51	0.51
Range	15-29	15-28	8-19	9-20	2-14	5-14
N	23		23		23	

REFERENCE GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Before	Diastolic After
1	29	19	15	10	9	6
2	17	19	9	10	5	6
3	17	15	10	10	7	6
4	27	25	15	13	8	8
7	17	19	9	12	5	8
8	19	20	11	12	7	8
10	22	25	14	18	8	11
13	13	25	6	15	3	9
14	21	27	15	18	9	7
17	24	28	13	15	8	8
19	15	15	10	10	6	6
20	20	17	14	9	8	5
21	22	20	13	12	7	7
22	12	14	–	–	5	6
23	19	20	14	12	8	7
24	14	13	9	9	5	7
25	27	19	11	12	7	5
27	24	18	16	12	10	8
28	16	17	9	11	5	9
29	27	30	18	19	12	11
Mean	20	20	12	13	7	7
SEM	1.13	1.09	0.71	0.70	0.47	0.38
Range	12-29	13-30	6-18	9-19	5-10	5-11
N	20		19		20	

Table XXIII. *Pressures in the pulmonary artery (PA) (mm Hg) during exercise before and after the training or reference period.*

TRAINED GROUP

	Systolic		Mean		Diastolic	
Case	Before	After	Before	After	Before	After
1	33	38	24	26	18	19
2	47	40	37	31	25	20
4	54	40	43	30	27	18
7	77	23	15	14	10	8
8	33	29	20	22	13	15
10	48	42	37	27	23	18
13	43	39	26	25	14	17
15	48	45	35	35	22	25
18	45	35	33	24	23	16
19	48	52	28	36	13	23
21	70	73	52	53	30	35
22	68	80	58	58	30	32
23	46	47	38	37	29	27
25	50	40	35	27	16	16
26	37	38	27	26	18	16
27	62	46	46	36	30	22
29	55	56	40	40	30	28
30	67	70	47	50	32	38
31	38	36	24	28	18	18
32	60	60	40	42	25	28
33	42	36	28	28	16	18
34	44	54	32	38	22	20
Mean	48	46	35	33	22	22
SEM	2.49	3.05	2.25	2.26	1.43	1.54
Range	27 70	23-80	15-58	14-58	10-32	8 38
N	22		22		22	

REFERENCE GROUP

	Systolic		Mean		Diastolic	
Case	Before	After	Before	After	Before	After
1	66	70	46	48	32	34
2	33	45	18	32	11	21
3	58	50	44	39	28	20
4	49	44	30	31	22	21
7	23	32	14	20	9	10
8	36	43	24	28	14	16
10	38	37	28	26	20	16
13	18	38	12	26	7	18
14	46	40	32	28	21	18
17	43	41	31	30	22	1
19	38	35	24	22	18	16
20	55	36	45	24	35	16
21	46	40	39	25	17	10
22	26	31	17	18	11	12
23	45	55	37	36	25	22
25	46	60	35	44	20	3
27	54	50	42	40	28	24
28	30	30	20	22	12	13
29	64	60	50	46	34	32
Mean	43	44	31	31	20	20
SEM	3.06	2.51	2.67	2.07	1.94	1.61
Range	18-66	30-70	12 50	18-48	7 35	10-34
N	19		19		19	

Table XXIV *Pulmonary capillary mean venous PCV pressures (mm Hg) at rest and during exercise before and after training or reference period.*

TRAINED GROUP

Case	Rest Before	Rest After	Exercise Before	Exercise After
1	8	7	15	18
2	8	9	23	18
3	5	8	–	–
4	16	9	37	27
7	2	4	5	7
8	9	9	14	16
10	8	5	37	25
13	7	7	–	–
15	6	7	29	28
18	8	5	20	16
19	8	10	23	23
21	–	–	40	40
22	10	8	45	45
23	7	6	26	27
25	7	6	25	22
26	6	5	–	–
27	9	5	28	20
29	8	4	25	32
30	12	6	32	30
31	11	10	17	22
32	7	9	–	–
33	8	11	24	18
34	9	6	20	28
Mean	8	7	26	24
SEM	0.57	0.44	2.25	2.02
Range	2-16	4-11	5-45	7-45
N	22		19	

REFERENCE GROUP

Case	Rest Before	Rest After	Exercise Before	Exercise After
1	8	4	46	45
2	4	7	15	25
3	9	10	33	33
4	6	6	19	20
7	7	8	8	17
8	8	7	12	17
10	8	10	19	16
13	1	6	11	17
14	5	6	11	13
17	4	5	15	18
19	6	5	15	16
20	9	4	28	10
21	5	6	17	13
22	4	7	10	9
23	9	5	24	28
24	8	6	24	24
25	11	10	–	–
27	10	8	36	36
28	4	8	10	18
29	12	7	46	24
Mean	7	7	21	21
SEM	0.62	0.41	2.71	2.12
Range	1-11	4-10	8-46	9-45
N	20		19	

Table XXV *Pressures in the systemic artery (SA) at rest before and after the training or reference period (mm Hg)*

TRAINED GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Before	Diastolic After
1	195	170	150	135	110	100
3	110	140	80	90	60	70
4	150	130	100	90	80	70
7	130	160	100	120	75	100
10	120	110	85	80	70	65
13	135	150	100	110	75	80
15	120	120	85	90	65	70
18	145	125	100	80	75	60
19	130	125	85	85	60	60
21	120	115	90	90	70	70
22	110	110	80	80	60	65
25	155	140	110	100	80	80
26	130	135	90	90	70	70
29	120	125	90	95	75	75
30	140	145	100	105	70	80
31	145	130	95	95	70	60
32	150	150	100	110	70	80
33	130	110	90	80	70	60
34	140	140	105	95	80	70
Mean	136	133	97	96	73	73
SEM	4.51	3.92	3.55	3.40	13.2	11.8
Range	110-195	110-170	80-150	80-135	60-110	60-100
N	19		19		19	

REFERENCE GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Before	Diastolic After
1	160	155	100	110	70	75
2	145	150	95	100	70	70
3	145	165	100	120	85	95
4	130	135	100	100	75	80
5	135	130	100	100	80	70
7	210	185	150	130	100	100
10	130	150	100	110	80	90
13	140	170	95	110	70	80
14	130	125	100	100	80	80
17	140	140	100	100	80	75
19	120	130	100	100	75	80
20	120	130	95	90	70	70
21	100	140	115	95	80	70
22	130	130	95	95	75	75
23	135	120	110	90	85	70
24	120	130	90	95	70	70
25	140	185	95	130	70	90
27	150	165	90	105	60	65
28	135	150	90	100	70	75
29	135	165	90	110	70	70
Mean	138	148	101	105	76	78
SEM	4.76	4.41	2.97	2.56	3.09	2.13
Range	100-210	120-185	90-150	90-130	60-100	65-100
N	20		20		20	

Table XXVI. *Pressures in the systemic artery (SA) during exercise before and after the training or reference period (mm Hg)*

TRAINED GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Before	Diastolic After
1	240	210	165	135	110	95
4	175	170	125	110	90	70
7	160	185	110	130	85	110
10	160	145	110	95	80	70
13	150	180	110	130	75	90
15	190	175	130	120	90	85
18	195	160	105	95	85	60
19	185	170	105	95	70	60
21	160	150	110	110	85	85
22	150	160	110	110	90	85
25	210	180	135	120	90	80
26	150	150	110	100	75	70
29	175	170	125	100	90	90
30	200	220	135	140	95	100
31	220	200	140	125	95	90
32	200	160	140	115	100	80
33	170	130	110	90	80	70
34	200	180	140	115	105	85
Mean	183	172	124	113	88	82
SEM	6.15	5.36	3.83	3.67	2.46	3.16
Range	150-240	145-220	105-165	90-140	70-110	60-110
N	18		18		18	

REFERENCE GROUP

Case	Systolic Before	Systolic After	Mean Before	Mean After	Diastolic Bef re	Diastolic After
1	195	235	130	165	100	120
2	185	190	110	130	75	80
3	180	175	–	–	110	100
4	160	170	120	110	90	80
5	160	175	110	120	80	100
7	255	225	165	135	115	105
10	160	180	120	130	95	100
13	200	200	100	140	80	100
14	170	150	125	120	100	80
17	180	180	125	125	95	90
19	160	160	130	115	90	85
20	145	180	110	115	80	80
21	200	200	140	125	90	85
22	160	150	110	110	80	80
23	170	180	125	120	100	90
24	175	170	125	130	90	100
27	190	220	120	130	75	80
28	180	160	110	110	75	80
29	250	270	160	180	110	120
Mean	183	188	124	128	91	92
SEM	6.62	7.12	4.00	4.35	2.88	3.04
Range	145-255	150-270	100-165	110-180	75-115	80-120
N	19		18		19	

Acta Medica Scandinavica

Supplementum 549

Ischaemic Heart Disease in Women

A study based on a randomized population sample of women
and women with myocardial infarction
in Göteborg, Sweden

By Calle Bengtsson

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica.*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each annually) include free supplements to the current volumes.

Subscription Rates

Per annum – two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw cr 170, incl. postage.
Other countries: Sw cr 200, incl. postage.

Chief Editor

Professor Jan G Waldenström, MD
Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Kungsholmsstrand 175
S-112 48 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell
Informationsindustri AB
S-751 81 Uppsala, Sweden

FROM THE DEPARTMENT OF MEDICINE
AND
THE DEPARTMENTS OF CLINICAL CHEMISTRY
OBSTETRICS AND GYNAECOLOGY AND PSYCHIATRY
UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

Ischaemic Heart Disease in Women

A study based on a randomized population sample of women and women with myocardial infarction in Göteborg, Sweden

By Calle Bengtsson

in collaboration with
P Björntorp G Blohmé, T Hällström, G Rybo E. Tibblin,
G Tibblin, J Waldenström, H Westerberg and L. Wilhelmsen

GÖTEBORG 1973

Printed I offset by Elanders Boktryckeri Aktiebolag

Kungsbacka 1973

CONTENTS

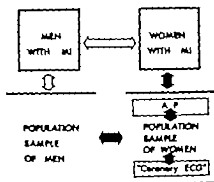


Fig 1 Possible comparisons (arrows) within the material discussed in the present report Filled arrows denote compari sons made in the present in vestigation AP angina pectoris coronary ECG ECG changes suggestive of IHD

population between men with MI and women with MI and betwe n subjects with different manif stations of IHD and the g neral population As shown by th fill d arrows in Fig 1 the present study of IHD in women aims to com pare women with and without IHD in the population sample to compare women with MI register d in th MI register and women in th population sample and to compare men and women in the respective population samples in order to find answers to two questions

1 Are there diff r nces betw en women with MI or other manifestations of IHD in the general population and healthy' women in th same g n ral population?
2 A e th re diff r nces in the pr valence of risk factors between m n and women in the g n ral population which can explain the lower incidenc of IHD in women?

Referen list pp 122 128

Chapt r I

MATERIAL AND METHODS

Th data of th p esent monograph d rive from a population study of women in Göt borg[15] nd from th Po t MI Clini in Göt borg[30,31] Göt bo g i th s cond larg st ity in Sw d n It i situat d on th w st coast and had about 445000 inhabitants in 1968 Furthe details conc rning Götebo g ar given elsewh e[182]

Th Population Study of Wom n in Göt bo g 1968 1969

P rformanc Th ba ic data of th present inv stigation w obtained from a population tudy of women pe form d in Göt borg Swed n in 1968 1969[15] A sampl of women in th ag strata 38 46 50 54 and 60 (born in 1930 1922 1918 1914 o 1908) totaling 1462 women wa tudi d Women born on dates which w ven multipl of ix (6 12 18 24 30) we called fo th study In the ag group 54 only tho who w born on th 6th o on th 12th of very month we call d and in the ag g oup 60 only thos born on th 6th The sampl was btain d from th R venu Offi Regist r Tho e born at th beginning of th ye r w e call d fir t Th survey wa performed fo th most part during a 12 month period In thi way th influ nc of ag diff ren within each ag g oup wa redu d a far a po sible

Th participation rat of the various ag groups are hown in Tabl I Th ov rall participati n rat wa 90 1 %

The examination of the population sampl took plac at Sahlgren Ho pital in Göteborg Th re a ch taff wa th me during the wh l examination period Th w men cam fasting in the morning and were interview d and examin d at diff rent examination station a ording to a po ial s hedul A tudy of cardiova cular di a wa on of th main r search proj ct of th investigation Ha matol gical gyna ologi al and psy hiatric studi w also performed as w ll a a proj ct on p clini al diabet s Th sam examiner perform d th sam examination during th whol tudy pe iod A more ompl t li t of search p oj cts is given l wh r[15]

Non pa ti ip nt Th number of refus and oth non participants is hown in Tabl I Singl women we e to ome ext nt ov r rep sent d among th fus s whil th w no diff n of importan between the participants and th fu e with rega d to pla of birth[5] T l phon int rvi w with most non participant t g th with studi of r ord f om inpati nt and outpati nt linic gave furthe inf rmation bout 93 % of th fu [11] In thi way information wa obtain d from altog th 99 2 % of tho who w still living in Göt bo g at the time of the examination It wa found that th re w n diff en of importan betw en the parti ipant nd th refu s with reg d t h rt dis [11] or blood pre su [?]

Tabl I Participants and non participants in th Population Study of Women in Göt bo g 1968 1969

Age (y ar)	Call d for examination	Non participants Dead	Moved	Inacce s ibl	R fus d	Parti cipants	Partici pation rate (%)
38	407	3	7	1	24	372	91 4
46	479	0	8	2	38	431	90 1
50	436	3	4	0	31	398	91 0
54	203	2	0	1	20	180	88 6
60	97	0	1	0	15	81	83 5
Total	1622	8	20	4	128	1462	90 1

Cau es of death 1950 1969 Death c rtificate wer obtain d from the Central Bureau of Statistic Stockholm fo all women who had di d in 1950 or lat r and who becaus of th sampling m thod would have been invited to the examination if still aliv Forty ight women had di d during th period 1950 1967 Anoth 8 wom n died in 1968 1969 during the interval betw en the sampling and the int nd d examination date Th diagnoses of these 56 wo men ar shown in Table II Th diagnosis wa based on autopsy findings in at lea t 38 of the women (68 %) Only a small number had died from heart di ea es This agr s with obs rvations from a previou similar study of cause of death in men[187]

Table II Cau s of death of 56 women who di d betw en 1950 1969 and who becaus of th sampling method would hav been invited to tak part in th population study if still alive

Cau of death	n	Cau of death	n
H art dis as	6	Liv r dis as	1
Aortic disease	1	Ileu after child birth	1
C eb al va cula di ea e	2	Myoma of th uteru	1
Thrombo mboli disea	2	Tuberculo i	1
Malignant hypert nsion	1	Sy t mi ollagen disea	1
Kidn y di as	5	Nervou system di ease	4
Diab t s	2	Suicid	8
Canc	15	Accid nt	4
Leuk mia	1		

Ischaemic heart disease in the population sample

Myocardial infarction The diagno is of myocardial infarction (MI) was considered e tablished wh n it had been ve ified in hospital

Angina pectoris Symptoms of angina pectoris (AP) were r corded by int rvi w according to the questionnaire proposed by Rose[155] AP was d fined according to Rose[155] as chest pain or discomfort with the sit including ith r the ternum or th left arm togeth r with the left ant ior chest This pain is provok d by ither hurrying or walking uphill (or by walking on the level for those who nev r att mpt mor) When it occurs on walking it should make th subject eith r stop or sla ken pac unl ss nitroglycerin is tak n It should disappear in th majority of occasions within 10 minut s or l ss from the time that th subj t tands still

ECG changes uggestive of is haemi h art disease (IHD) An ECG examination wa performed aft r over night fasting with the subject in the supine position on a coach. A trained nurse took the ECG ft r th subject had been resting for about 10 minute A dir ct writing r ord r (Mingograf 42 B El ma Schönande AB Stockholm Sweden) was u ed Leads I, II III aVR aVL aVF CR1 CR2 CR4 CR5 and CR7 wer recorded The ECGs wer interpr ted by an experi nced techni ian (Mr Dana Vrobova Pragu) using th Minn sota Cod [14] with th Scandinavian modification[7] with out acc ss to othe information about th subj cts Minnes ta Codes 1 1 2 4 1 5 1 2 (in the ab en of 3 1) 6:1 7 1 were defin d a coronary ECG

Th Po t MI Clinic in Göteborg

Registration Subje ts born in 1913 or lat r who live in Göteborg and who have had an acut MI have be n regi t ed at th Post MI Clinic sin e the 1 t of January 1968[59] Th r gistration has been carried out unde th sup rvision of the Section for Pr ventive Cardiology at Sahlgren s Hospital Göteborg Th intention has been to gist all men and women with an acute attack of MI

Subj cts having a MI in Göt borg are tak n to th m rgency departm nt of Sahlgr n s Hospital which is th only hospital for acute medical care of heart diseases in Göt bo g An initial registration is made at th mergency depart ment All subj cts with suspected MI are followed up by the regi tering per onn l until the diagnos s of MI is proved or di prov d In addition all medi al wa ds ar visited by r gi t ring per onn l twic a w ek in order to r gista subj cts who diagnosis of MI might hav be n mi ed at th mergency d partment

C t ia of MI Th it ria of acute MI have be n:
Central best pain of more than 15 minut du ation with onset during 48 hou and/or frank pulmonary oed ma without pr viously kn

h art di ease and/or shock without suspicion of acut hypovola mia or intoxi cation together with

a) app arance of a pathological Q wave and/or appearance or disappearanc of a localiz d ST elevation follow d by a T inversion in two or more of l. l ads

and/or

b) two SGOT values above the upper limit fo normal variations of th labora tory[17] with a maximum level about 24 hours after on et of symptoms in combination with low r SGPT values with a maximum level after about 36 hours

The criteria agr e with those r ommend d by th Swedi h H art Society

The egistration of the subje ts with symptomatic MI has been supplemented by studying all death certificates issu d sinc th 1 t of January 1968

Further d tails about the r gistration of symptomatic MI incidence of MI and incidence of de th from IHD are given elsewhere[0]

Performanc of the int rvi ws and xaminations Th sub j cts with MI w r int rview d and examin d in a standardi ed way by on of fiv phy ician ac ording to pecial form Th m thodology was generally th sam as in th population studi s of men[1,2] and women[15] in Göteborg When th re ar differ nces in methodology the will be mention d in the s parate chapters The women w e examined at th time of the acute attack and at linical visit 3 12 and 24 months after the atta k[51] In addition the women w r examin d by th autho on an extra occasion in ord r to obtain omplem ntary and suppl mentary data

Wom n with myocardial infarction registered at the Po t MI Clinic

Su vivors on arrival in ho pital Th p e ent inve tigation con rns women in Göteborg who we e born in 1913 or later and who had an cut attack of MI during the y ar 1968 1970 Tho who l ft ho pital as survivors we e admitt d to th tandardiz d follow up study with linical vi t 3 12 and 24 month after the MI One woman ag d 23 with sy t mic lupu rythematosus had been ex lud d from the study as h r MI was con d ed to be a complicat on of her pre existing dis ase[13]

Death The d ath c rtificates of all women in Göt borg who wer born in 1913 or lat r and who had di d during the years 1968 1970 w r examined Further data about thos dead we obtained from inpatient r cords and autop y report

Women who died suddenly (d ath within th first f w hours befor reaching hospital) were ons d ed to have di d from MI when autopsy rev aled a fre h MI ar o when acut s vere chest pain had pre ded death[17]

Unexpected d aths were classified as co onary deaths wh n th women were not known to have had che t pain pr eding death, and autopsy reveal d sev re coronary athe oscl rosis but no fr sh MI area [II]

Pr vious studies on m n

A population tudy of 855 50 year old men in Göt bo g was carri d out in 1963[181] and these men we e re xamined in 1967 wh n th y wer 54 years old Th men w r representative for th gen ral male population in th same way as th women in th present study w re representative for th gen ral f male population in Göt bo g Consequently omparisons may be made between men aged 50 and 54 and women in the present study aged 50 and 54 in ord r to look for differ nces between men and wom n in th g n ral population of these age groups

Method for comparing women with IHD and women in the gen ral population

Th parametric variabl s of the women in th population sampl were di vided into dec ntil wh n data from all the ag groups tudied w re available Th women with IHD w then pla d in th d entiles of th se variabl s a cording to th d ntil limit for their ag in th population tudy In thi way a omparison ould be made betwe n th wom n with IHD and th wom n in th population sampl Th whol population sampl then constitut d the ref renc group Comparison ould al o be mad with th g neral population as the population sampl was r pre entative for th general f male population. The valu s of th women with IHD were expected to li within the upper d centil s if th re was an as ociation betw n IHD and high valu s of the variable tudi d

The non parametri variables of the women with IHD wer usually compar ed with tho of th wom n of the ag s 50 and 54 in the population sampl who had about th sam mean g as th women with IHD Tabl III how mean ag standard d viation (S D) nd rang in th diff nt g oups of women with IHD and in th group of women from th population tudy ag d 50 and 54 who thus usually onstitut d th ref renc group wh n studying th non parametric variables Fo special rea on oth r referen group were chosen wh n tudying glu o tol ran and in ulin respon to intravenous glucos inj ction[III] and when studying str s factor and behavioural trait [I] The e exc ptions will be di cuss d parately in th chapt rs which on ern these matt

The compari ons were mad betw en th women in th diff rent IHD groups and tho in th refe en group who w re fre from the IHD manifestation tudi d.

Tabl III Mean age S.D and range (years) in th groups of women with MI AP and coronary ECG and in the participants of th population tudy aged 50 and 54 who usually constituted the reference group

Cat gory	n	M an age	S.D	Rang
Women with MI				
Survivors on arrival in hospital	47	52 2	4 5	37 57
Deaths outsid hospital	10	50 5	5 3	40 56
Women with AP	29	51 3	4 8	38 60
Women with coronary ECG"	23	54 2	6 8	38 60
Women aged 50 and 54 in the population sample	578	52 0	2 2	50 54

Stati tical methods

Conventional methods we e u d for calculation of mean values S D and standard error of th m an (S E) Significan e of differences between mean value wa stimated with Student s t test (two tail d t st)[31] Th hypoth sis of differences in frequenci s between groups was te t d by means of th binomial distribution with a normal approximation[31 159] Th following formula was used

$$\frac{P_1 \qquad P_2}{\sqrt{P \times (1 \quad P) \times (\frac{1}{n_1} + \frac{1}{n_2})}}$$

wh re P_1 is the proportion of women with a certain chara teristic in on of the IHD groups and n_1 th number of women in this group P_2 th proportion mong thos in th r fe enc group who do not hav th same IHD manif sta tion and n_2 the number of women f e from th IHD manife tation studied in thi ref ren group P i the pooled proportion of P_1 and P_2 according to th formula

$$P \qquad \frac{n_1 \times P_1 \qquad n_2 \times P_2}{n_1 + n_2}$$

If the null hypoth s ould be je t d th following alternativ hypothesis w s ac ept d. a rtain haract risti is more common in women with IHD than in women in the gen ral population Thus a on tailed test was applied with a few x ption (when th variabl studied was not xpected to be mor common n on group than in th oth ac o ding to previou tudi of men or wom) If a two ta l d t st w us d thi i discus d pa at ly in the vari ou hapt rs

Wh n testing th hypothesi that the valu s of the women with IHD hav the am central t nd ncy as tho of th women in the gen ral population th following formula was u d

$$\frac{P_1 \quad 0\ 5}{\sqrt{0\ 5 \times 0\ 5 \times \frac{1}{n_1}}}$$

wh P_1 is the proportion of women in one of the IHD group with valu abov the median of the population sampl and n_1 the number of wom n in thi IHD group The alt rnative hypoth sis was women with IHD have valu high r than tho of women in th g n al population (one tail d te t)

Th diff nc s w considered stati tically ignificant for $p < 0\ 05$ Th data from the population tudy w r analysed by means of a omputer (IBM 360/65) Th data from the women with MI were usually t t d by m ans of a d k alculato (H wl tt Pa ka d 9100 A) Th tatistical analys s of str s and behavioural traits[I] w r carri d out by Mr Ande s Odén Mrs Gull britt Palm was onsultant statistician and wa r sponsibl for th comput r analy is of all th oth r variabl tudi d

COMMENTS

Th population ample B au of th method of l tion the popul tion ampl i on id d to be repre entativ for th g n ral population of women of th ag tudi d Th high participation rat ensu s that th re ult obtained in th tudy ar pr entativ for th sam population. T l phon int rview with mo t non parti ipant tog th with th information obtained from inpati nt and outpati nt o d furth r onfirms th as rtion that th results obtain d ar ep ntative fo th gen al population of wo m n in Göt bo g

Wom n with AP and co onary ECG A th women with AP and o onary ECG w pa ti ipant in th population tudy they were re pr ntativ fo th women of imila ag with th s manif tation in th gen l popul tion

Wom n with MI u vivo on a ival in ho pital Th MI g oup i believ d to includ almo t all women born in 1913 o lat r who had a symptomatic MI during th period Pr tically all peopl with symptoms of cut MI in Göt borg go dir tly to th mergen y d partm nt of Sahlg en Hospital If a doct i a ked to th pati nt at h me thi docto will imme di t ly tran f th patient to the ho pital Th condition p obably som what diff rent f om thos in oth ountri s g in U K and U S A wh re ubj t with MI mo often m to be ca d fo at h m[6 01]

In o d to heck th ompl ten of th MI r gi t during 1970 th

diagnoses of the patients in departments other than those of internal medicine were studied e g the departments of allergology neurology and surgery[50] Subjects with MI born in 1903 or later were registered during this year In all, 390 subjects were then regist red and anoth r 13 subjects (3 2 %) with an acute MI were found to have b en cared for at departments other than those of internal medicine However no woman born in 1913 or later was among these 13 subjects with MI[50]

Interviews and when ne ded supplementary studies of hospital records of 4734 men in Göteborg born in 1915 19 0 revealed that of 33 men with an acute MI during the years 1968 1970 had not been registered in the MI regist r[50] Another previous study on the occurrence of non hospitalized cases of suspect or definite MI revealed that only one of 161 survivors remained at home in spite of a clinical MI[61] Taken tog ther thes observations guarantee that the MI group is almost complete with regard to the number of women with clinical MI The low primary mortality[II] and the high pa ticipation rate in the follow up study nsur d valid data on women who suffered MI without an immediately fatal outcome At the standardized clinical controls 3 and 12 months after the MI 38 (90 %) and 41 (98 %) of 42 survivors participat d In addition all the 42 survivors were interviewed and examined by the author on an extra occasion

The number of non symptomatic MI in Göteborg is not known Non of 1462 women participating in the population study had ECG abnormalities denoting pr vious MI (Minnesota Codes 1 1 2) which may suggest that silent MI is infrequent in women in Göteborg[II]

Women with MI deaths outside hospital MI resulting in sudden death was almost always recognized as 80 % of women who died from cardiovascular disease outside hospital in Göteborg were autopsied[50] In addi tion some women had a history of severe ch st pain pr c ding death which strongly suggests an acute MI[II 16] Th number of deaths cla ified as MI or coronary deaths probably includ s all women in the ages studied who died from IHD during the period. However as th study was not pro pe tive comprehen sive valid data w r mostly not obtained on the women who died outside hospital

Validity of the diagnoses of MI AP and coronary ECG The rit ria of MI in th pres nt study ar those proposed by the Swedish H art Society and hav be n used previously[83] There was good agreement between these riteria and the clinical impression of the physicians who treated the patients during their stay in hospital

It is mor doubtful as to wh t ext nt th riteria of AP and coronary ECG discriminated subject with IHD The criteria of AP according to Rose[155] have been widely us d and they have in pr vious studi s been found to have a high specificity and sensit vity when compared to physic ans diagnoses on clinical grounds[8 59] They w re ns dered to discrimi at subjects with AP fairly

well in th pres nt tudy[II] though they wer probably l ss reliable than those of MI The ECG crit ia of IHD us d in the p sent tudy[I] have previously been u d in for instance T cumseh U S A[57] and in Busselton Australia[196] Co exist nce of AP and ECG ha g s denoting IHD w re v ry uncommon in both studie as w ll a in the pr s nt study (Table IV)

Tabl IV AP and coronary ECG as ingl manif stations and in combination (n) Th studie in Tecums h U S A[57] Bu selton Australia[196] and the Study of Women in Göteborg 1968 1969

	Only AP	AP + Co o nary ECG	Only Coro nary ECG
T cums h[a]	9	1	22
Bu s lton[a]	6	1	16
Göt borg[b]	26	3	20

[a]Women ag d 40 59 [b]Women ag d 38 60

Minn sota Cod 4 1 and 5 1 2 were th predominating ECG manif stations in women both in Tecumseh and Busselton, while Minn sota Code 1:1 2 wer common in men In th p s nt tudy Minn sota Code 4 1 and/or 5 1 2 were r co d d in ll th women with co onary ECG and Minn ota Cod s 1 1 2 in none It s m that th ST d p ssion od d as Minn sota Code 4 1 and the n g tiv o diphas T amplitud coded a 5 1 2 (ven in th ab enc of high R amplitude oded a 3 1) e not specifi fo IHD in wom n The vast majo rity of th wom n with coronary ECG in th pr s nt tudy had art ri l hyp tension[II,V] It ther fo m p obable that coronary ECG chara t ri ed by Minnesota Cod s 4 1 and 5 1 2 s a manif tation of hypert nsiv di s e rath r than IHD in wom n[II,] How v the po sibility that o on ry ECG could be a pr symptomati manif tation of IHD in hypert n ive ubj cts ha al o to b con id r d[V]

Cardiomyopathy of va ou origin has be n reported to u ECG chang s[125] but i probably not a ommon ontributor to ECG hang s in women in Sw d n

R tro p t ve tud e ontra a e ont of tudi A p o spe tiv tudy s ms to be th ide l way to obtain information about th sso c tion betw n diff t vari bl s and IHD Pr morbid dat a mor valid a th di a per may influen the vari bl s if th s tudi d t th time of o afte th on t of ymptom How ver v n in prospective tudi uch a th in F amingham U S A[97] nd in T ums h U S A[57] th num be s of wom n who uff r d IHD during long-t rm follow up p l lly th numb r of w men who had MI w re quite m ll Th Framingham Study com pri d 2844 women g d 30 59 nd th T cum h Study 2157 wom n ag d 16 or

old r who we free of IHD on entry to the studi s The wid ange of the age group in thes studi s makes th interpr tation of th r sults ev n mo e diffi cult

Th low incid nc of MI in young and middl aged women r quires v ry larg series in o d to be able to carry out valid tatistical analysis In Göt borg with an annual incid nce of MI 0 39 p 1000 women ag d 50 54 including sudd n d ath 0 56 per 1000[III] about 40 (in luding sudd n death about 60) women would be expect d to d velop MI within 5 years if ~0000 50 y ar old wom n were in lud d in a p o pective study Thu a very large mat rial and/ or a v ry long follow up period would be n cessary Th pr s nt method of tudying as oci tion b tw n diff r nt variabl s nd MI is not con id r d to b id al but is probably th be t on in ord r to rec ive an answ r within a reasonabl tim

How ver when carrying out a cas control study it is n s ary to be awar of it limitations Systemati differenc s b twe n th affli t d subjects and their cont ols and influenc of the dis ase studi d per s s em to be the two main limiting facto s

R f r nce group in th pr s nt tudy When possibl th total population sampl was u d as a ref renc group This was u ually poss ible when studying pa ametri variabl s It wa not po s bl to study non param tric variables in th s m way The women in the age strata 50 and 54 had about the same m an age as the women with IHD but it is to b emphasi ed that th ag distribution wa not th same in the women with MI and in tho e with oronary ECG th wa ome accumulation in th upp r ag trata whil the age di tribution of th women with AP was similar to that of th ref nce group[II,II] Th diff r nc in ag d stribution betwe n the wom n with MI and th eferenc group must be taken into consid ation pecially when the variabl xpe t d to chang markedly with g during th sixth decad Fo example one of the vari bles tudi d wh n discus ing the po sible influ n of str ss facto s last child l ft home during th last y ar' was found to be most common n wom n ag d bout 54[I] When studying th str s facto s wom n ged 54 had been cho en a a r f ren e group It wa found that the l st ch ld had left hom during th last y ar more often in the r f ence group than in th MI group but thi might be explained by different age distribution n the two g oup Simila ly a slightly higher pr val nc of diabet s mellitus may be xplained by the fa t that the pr val n of diabete mellitu in r ases pid ly w th g How ver when th diff ren betw en th wom n with MI and the women in th r f ren group was a prominent as fo diabet in th pr s nt study th ag f to s could b rul d out Smoking might be expected to be l common in the wom w th MI a moking s l ss ommon in older women If the oppo it s found a w th cas in the p nt study[VII] th diff ren in

age distribution give further strength to th finding

It is difficult to have a perf ct ref renc group in case control studi s lik the present one The gr at advantage of th pr sent fer nce group is the fa t that the women in th e groups are r pr sentative for th g neral population of women in the same way as the women with IHD are repres ntativ of the women with IHD in th same gen ral population Th diff rent ag distribution must be taken into consideration wh n studying the non param tric variables

Statisti al analys s of non param tric variabl s were u ually carried out on women with a manif station of IHD and th se in the reference g oup without this manif station For example a compari on was mad between women in the AP group and tho e fre fr m AP in the r f r nc group None of the parti ipants in th population tudy g d 50 or 54 who usually provid d the ref renc group for comparisons of non param tric variabl s had a history of MI Only on of them suff r d MI during th pe iod 1968 1970 H r influence on th re f renc group as a whol could be negl ted and sh was th r fore not exclud d from th refer n group when carrying out compari on between wom n with MI and women in th ref renc group Th compari ons of pa ametri vari abl s between women with and without IHD w re usually ref rr d to th median values of the total population ampl A the women in the IHD g oups could only have a minimal influen e on thes valu in various ag group thi poss ible influen was n gl ted, and the omparisons we always ferr d to th median values of the total population ampl including the women with AP or co onary ECG Thi simplifi ation ould n t bia the stati tical analysi

Influ nce of MI or AP per e on variou variabl An acut MI may d crea th blood p ssur [7] and reduc the s rum lipids [II] Ch t pain in ubject with AP may r du e the physi l a tivity and thi in turn may have ondary eff cts As AP is a common f ature in subj ts who uff r n acut MI [II] h t pain and its consequ n es may have influen d a numbe of variabl v n befo th MI attack. Su h facto may c u diffi culty when interpr ting th results and have be n di cuss d in th variou chapte s Efforts have b n mad to rul them out a much a possible For in tan hi tory of hyp rt nsion and family hi tory of hypertension have be n studi d in tead of th blood p su value in women with MI chol t rol and triglyc rid measurem nt hav been ca ri d out on s ve al occasion [II] and m ntal tress wa m asu d as exposur t rtain defin d stre s fa tors in addition to the w man s report of subjective xperien of stre s[I] In this way th influ n e of the MI per i onsid red to be liminated as much as possibl but th di cu s on i n arily limit d when the l lack of pre mo bid data

Possible influen e of interobs rv r variation The examina tions and int rview in th population study wer pe form d by th same exa min rs As th wom n with AP w r participants in th population study th y wer tudi d in exactly th sam way as the oth r participants The examina tion and int rview of the wom n with MI wer carri d out by a team of physi cians whi h makes interobs rver variation p obabl within th MI group and betw en th women with MI and the population sampl Howev r int robs rver variation is not onsid r d to be an important ource of error in the pr sent tudy

Similarly the population studies of men and wom n w carried out by diffe nt examiners Ther were al o som other diff renc s The tudy of 50 y ar old men was carri d out in 1963[182] and th m n w re r examin d in 1967 Th fir t tudy of the m n wa thus performed about 5 y ars b for th study of th women The importanc of thi tim differ n e is unknown but is p obably not great Som dis pancies in m thod of xamination hav been discus d parat ly[XIII] However a a whol a similar m thodology was app li d to both tudi s In addition both amples are r p entativ of th sam g n al population and thi i r garded as th most important point

Con id rations on th sign fi ation of risk factor

Risk fa tor is a commonly a c pted expr sion in pid miologi al r s ar h Th exp es ion is to som d gre mi leading a it may hint that th facto i al o a tiologic l A risk factor may be a tiologi al but is not n ces arily o It is important to mpha i that a risk facto m ans a det ctabl attribute or circumstanc of an individual which i known to be statistically a sociat d with an inc as d isk of dev loping overt mo bidity but nothing mo The facto n d not in t elf be es ntial for d veloping dis a e but may be tati tically a ociat d with anoth facto whi h may be an aetiological one Th f ctor may be of impo tanc only in combination with one or mor oth r known o unknown facto Int rvention aimed at liminating risk facto s th fore does not n s arily m an a d cr as d risk

Roman num l f to th hapt of th monog aph Arabi numeral f r to th r f nc li t pp 1 2 128

Chapter II

PREVALENCE OF ISCHAEMIC HEART DISEASE IN WOMEN IN GÖTEBORG, SWEDEN

Calle Bengtsson

Abstract. From a population study of middle-aged women in Göteborg, Sweden, the prevalences are given of history of myocardial infarction, angina pectoris and ECG changes suggestive of ischaemic heart disease. A comparison is made with men of the same ages living in the same area. The prevalence of myocardial infarction was found to be higher in men than in women, while that of angina pectoris was about the same in both sexes. Q-wave changes of the ECG were more common in men than in women, while other ECG changes suggestive of ischaemic heart disease, such as ST depression and T-wave inversion, were as common in women as in men.

A great deal of information about ischaemic heart disease (IHD) has been obtained from mortality statistics. Population studies have also given valuable information about various manifestations of IHD. The present communication deals with the prevalence of myocardial infarction (MI), angina pectoris (AP) and electrocardiographic changes suggestive of IHD ("coronary ECG") as found in a population sample of women representative of the total number of of women aged 38-60 in Göteborg, Sweden.

MATERIAL AND METHODS

The data were obtained from a population study of 1462 women carried out in Göteborg during 1968-1969[1,5]. All participants were questioned concerning a history of MI and AP. AP was defined according to Rose[53]. The ECGs were interpreted according to the Minnesota Code[18] with the Scandinavian modification[9]. Minnesota Codes 1:1-2, 4:1, 5:1-2 (in the absence of 3:1), 6:1, 7:1 were defined as "coronary ECG".

Further details of material and methods are given in Chapter I.

RESULTS

Prevalence of IHD in women

History of previous MI. As seen from Table I there were only 2 out of 1462 women in the population study who had had a verified MI, one aged 46 and the other aged 60. Another 2 women had a history of severe chest pain lasting more than 30 minutes. They had not been hospitalized and their ECG did not reveal changes suggestive of MI.

Angina pectoris. As shown in Table I the prevalence of AP increased with age from 0.3 % in women aged 38 to 3.9 % in women aged 54. As the population sample contained so few participants aged 60, conclusions concerning the prevalence in this age must be drawn with caution.

Tabl I Prevalence of MI AP and coronary ECG
The Study of Women in Göt borg 1968 1969

Age (years)	Subjects at risk	Subjects with MI		Subj cts with AP		Subjects with coronary ECG	
	n	n	%	n	%	n	%
38	372	0	0	1	0 3	1	0 3
46	431	1	0 2	6	1 4	5	1 2
50	398	0	0	12	3 0	4	1 0
54	180	0	0	7	3 9	3	1 7
60	81	1	1 *	3	3 7	10	12 3

Coronary ECG 'Coronary ECG was record d in 23 women (Table I) The prevalence increased with age ST depr ssions coded as 4 1 according to the Minnesota Code w re recorded in 18 women and T wave changes coded as 5 1 2 in 22 wom n (5 1 in 3 and 5 2 in 19) Co existence of 4 1 and 5 1 2 was found in 17 women In addition Minnesota Code 7:1 (left bundle branch block) was recorded in 2 of these women. No ECGs with Q and QS pattern coded as 1:1 2 or complete atrio ventricular block coded as 6 1 were found Minnesota Cod 3 1 (high amplitude of R waves) was record d in 5 women who also had ECG changes classified as Minn sota Code 4 1 and 5 1

Fig 1 shows the co existenc of AP and coronary ECG Only 3 women were classified as having both AP and coronary ECG

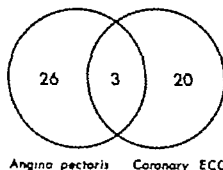


Fig 1 Co existenc of AP and coronary ECG Th Study of Women in Göteborg 1968 1969

Pr valen of IHD among th non attenders Information about heart diseas was obtained by int rview from 116 of 128 women (91 %) who refused to attend th population study and from records in another

3 women who had visit d the medical gyna cological or psy hiatr c servic s during th previous thre y ar In this way information wa obtain d f om 93 % of tho e who refu d to attend None of th m had had a verified MI and none omplain d of h st pain

Women who had di d in 1950 o later and who would have be n call d fo th population study if still aliv w re studi d by means of thei d ath c rtifi cat s Six out of a total of 56 had di d from dis a of th heart acco ding to the de th certificates[1] On of thes women had di d from cong nital heart di a e two from rheumati heart disea two f om IHD and on f om pa alyti heart' Th diagnos of th fi st mention d 5 women we ba ed upon autopsy finding

Preval n of IHD a compar d to other hea t di eas in women It was n t int nded to study in detail th prevalenc of various ong nital and heumati h art dis as in the population sampl How ve s me information was obtained from history and phy ical examination The prevalence of th h art di ord wa low (Tabl II) Four women had pre viou ly been diagno d a valvular heart diseas In 8 women murmu s w re found making heart di a p obabl The p val n of IHD was high than the p valenc of th oth r h art dis a s A port d above 2 women had di d f om IHD and from heumati h art dis a in 1950 among th e who would have been included in th mpl had they still been alive

Tabl II Prevalen e of m heart di ord
Th Study of Women in Göt b g 1968 1969

	n	%
V rified myoca dial infa ction	2	0 1
Angina pectori	29	2 0
Coronary ECG	23	1 6
V ifi d valvula he rt di prior to the examination	4	0 3
H art murmu sugg tive of ong nital o heumati heart di ae previou ly unknown	8	0 6

Compa i on b tw n th p val n of MI AP and o onary ECG in m n and wom n A ompari on betwe n the p valen of MI and AP in m n and women f om Göteborg i mad in Fig 2 A p pulation tudy of men born in 1913 had been pe form d in 1963 when th men w re 50 yea old[182] Th m n w examin d 4 yea lat at th g of 54[183] Th pr val n of AP was bout th sam in th two s x at th ag of 50 and 54 but the pr valen of MI was mu h high in th men

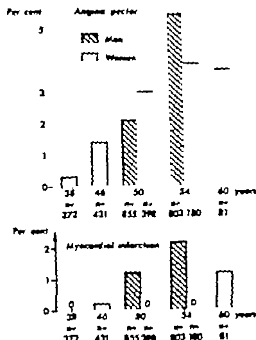


Fig 2 Prevalence of AP and MI in men and women in Göte borg. The Study of M n Born in 1913 [107,103] and the Study of Women 1968 1969

The preval nce of ECG changes suggestive of IHD could be compared b t ween 54 y ar old men [103] and women (Table III) Q waves (Minnesota Code 1:1 2) we e recorded in 1 6 % of th men but in non of the women while ST d pres sions (4 1) and inverted T waves (5 1 2) wer as common in the women as in th men

T bl III Pr val nc of ECG changes suggestive of IHD in 54 year old men (n 793) and 54 y ar old women (n 180) in Göteborg
The Study of Men Born in 1913 [3] and th Study of Women 1968 1969

ECG ategory Minn ota Cod	Men		Women	
	n	%	n	%
1 1 2	13	1 6	0	0
4 1	12	1 5	3	1 7
5 1 2	16	2 0	3	1 7
6 1	0	0	0	0
7 1	2	0 3	0	0

DISCUSSIO

Ach son Epstein d Tra y [88] have r viewed reports on mortality in IHD prevalenc d inc denc of IHD and sex rat o in diff rent ountries The sex ratio is diff r nt differ nt ra es and countries How ver th constant

finding of a higher prevalence of MI in middle aged men than in middle aged women in the industrial countries agrees with the results of the present study in Göteborg

Population studies have given limited data concerning IHD in women. Because of the differences in criteria, methods of sampling, age grouping and presentation, the results of the various studies are seldom comparable. However, in Table IV an effort is made to compare the data obtained in Göteborg with those from some previous investigations in men and women, viz. the population studies in Framingham, U.S.A.[42], Tecumseh, U.S.A.[57] and Glostrup, Denmark[72]. The wide span of the age groups in the presentations of data from Framingham and Tecumseh makes comparison with the Göteborg data difficult. The Scandinavian figures for MI are about the same as the corresponding U.S.A. figures. AP seems to be more frequently reported in Scandinavia, both in men and women. This difference may be explained by differences in criteria and in the manner of presentation of data.

Table IV. Prevalence of MI and AP (%) in men (M) and women (F) in the Framingham[42], Tecumseh[57], Göteborg and Glostrup[72] studies.

	Framingham					Göteborg				Glostrup			
Age (years)	MI		AP		Age (years)	MI		AP		MI		AP	
	M	F	M	F		M	F	M	F	M	F	M	F
30-44	0.2	0	0.2	0.4	38		0		0.3				
45-62	1.6	0.1	1.9	1.2	46		0.2		1.4				
	Tecumseh				50	1.4	0	2.1	3.0	0.9	0	4.4	2.1
40-59	2.5	0	2.0	1.2	54	2	0	5.5	3.9				
					60		1.2		3.7				

It is of special interest to compare the prevalence data from Göteborg with those from Glostrup, Denmark, as 50 year old men and women were studied in both places. There were no differences in the prevalence of MI or sex differences for MI (Table IV). The Glostrup study revealed a moderate sex difference for AP which was not found in Göteborg. The moderate or absent sex difference in the prevalence of AP is in agreement with the results from other studies[51, 57, 58]. Thus the prevalence of AP is about the same in men as in women, but there must be some factor or factors making men more liable to MI than women, as will be discussed further in Chapter XIV.

ECG changes classified as Minnesota Code 1.1-2 were more common in the men than in the women, while there was no sex difference concerning ST depressions (Minnesota Code 4.1) and T wave inversion (5.1). This agrees

clos ly with pr vious observations in Tecum h U S A [57] and in Buss lton Australia[196]

It i obvious from previous studi and from th pre nt tudy that all manif stations of IHD inc a with ag Conclusion about th pr valen e figure conc rning the 60 year old parti ipants of th pr s nt population study must be drawn with caution as ther w r few women in this age group

It i obvious that the number of women who had died f om IHD was small as shown when the causes of death w r studi d in tho who would have parti ipat d in th population study if till alive The low pr valenc of MI in wo men in Göt borg was thus not du to an ea ly death from thi cause

It can be d bat d whether th crite ia us d for th various manife tations of IHD really d tected tho e with IHD Only 3 women w consid red to hav both AP and co onary ECG whil most of th m had only one of th se mani festations (Fig 1) This may sugg st that the criteria are not suffici ntly pecific Similar ob rvation have been mad in oth r parts of the world [I] The standardi ed qu stion for AP were found to have a high specificity and sensitivity wh n compared with phy icians diagnos on clinical g ounds as hown in two p viou studie of m n [81 155] Even though the sensitivity and specificity in women are n t nec ssarily simila it s em probable that the standa di ed que tions will di riminate subj ct with AP fairly w ll

It is more doubtful whether the coronary ECG a d fined in th pre nt study really di criminat s women with IHD It was found that out of 23 women with oronary ECG 11 we on antihyperten ive treatment Anoth 2 were on diur tic for oth r asons Of the 10 women who w re not on anti hypertensive treatm nt or diur tics 8 had a ystolic blood pr sure above th median for th population sampl [V] Th ECG hang of the women with coronary ECG were ther fore su pected of being a manif station of hyper tension rath r than IHD[I]

Du to the high parti ipation rate and the oll ction of information about th non-attenders in whom no over representation of IHD was found the re sults obtained from the pr sent population tudy conc rning the prevalen e of IHD ar con ide d to b a valid a the criteria fo th various manif stations

IHD in women pecially MI i rar It should be noted howeve that th present population study reveal d a higher p val nce of IHD than of valvula h art disea in wom n of th ag studied Although less fr quent than in middle ag d men IHD in middle aged women is frequent nough to be of importanc

Roman numerals ref r to the chapters of this monograph Arabic numerals f to th ref nc li t pp 122 128

Chapt r III

INCIDENCE OF MYOCARDIAL INFARCTION IN WOMEN IN GÖTEBORG SWEDEN

Call Bengtsson

Abstract The annual inciden of myocardial infar tion in Göt borg Sw d n increas d from 0 03 per 1000 women ag d 35 39 to 0 39 in women ag d 50 54 including sudd n death from 0 05 to 0 56 per 1000 The inciden wa about 8 times higher in men than in women

Information about th incidenc of myoca dial infarction (MI) in men has been obtained from population studies Less is known about the incidenc of MI in women exc pt that it is low If it is to be tudied in a population sampl of young or middle ag d women the sample must be very larg or the follow up must continu fo a long time if reliabl information i to b obtained During a mean follow up period of about 4 year only 2 of 1462 women who participated in a population study in Göteborg Swed n in 1968 1969[I, 15] suff red MI In o der to obtain mo e valid information th inciden e of MI wa studi d in th t tal number of women living in Göt bo g who were born in 1913 o lat r

MATERIAL AND METHODS

Data were obtained from a MI r gister which included all subj cts (men and women) known to have had an acut attack of MI during the y a s 1968 1970 who lived in Göt bo g and we e b rn in 1913 or lat [I,90] A detail d report on th incidenc of MI and IHD death in men and women is given by Elmf ldt t al [90]

The population at risk consist d of all individual living in Göteborg who w re born in 1913 o late [171 172 173] Th total numbe of subj cts in diff ent ag groups who had a MI during the year 1968 1969 and 1970 e g tho aged 50 54 at the beginning of th years wa added up Simila ly the total numbe of individuals at risk in th same g groups throughout the th years was add d up and th mean annual numbe of subj cts with MI pe 1000 individual was calculated Th women who wer 55 y r old or old r at th beginning of th yea in whi h they had th ir MI o died w n t in luded when calculating the in iden e figur

Deaths outsid ho pital w e classifi d a MI when autopsy r veal d a fr h MI a ea o wh n ver hest pain pr ded d ath[I] Two women we l ssifi d as death out id ho pital although they w r in titution liz d for a one othe than h rt dis[II]

Furth r d tails of mat rial and method a given in Chapter I

RESULTS

Forty seven women with MI were survivors on arrival in hospital while 10 women died from an acut attack of MI outsid hospital The death of an other 4 women classified as coronary deaths was probably caused by an IHD attack Tho ag d 54 or younger are presented in Tabl I Tabl I also shows the annual incidence of MI and d ath outsid hospital from a probable IHD attack in women aged 54 or younger The annual incidence of MI in women in creased from 3 per 100000 in the ag rang 35 39 years to 39 per 100000 in th age rang 50 54 If non ac idental un xpect d sudd n death is included th se inciden es became 5 and 56 per 100000 respe tiv ly Of 12 women aged 54 or young r who died suddenly 9 were consid r d to have died from MI and 3 wer classified as coronary d aths

Table I Annual incidence of MI and sudden death (non accidental unexpected death within a few hou s) p r 1000 women in Göt borg born in 1913 or later (ge at the beginning of th year is record d th incid nce figures in brackets i clude sudden d aths)

Age group	Women hospi talix d becaus of MI 1968 1970	Sudden death outside hospital from probable IHD 1968 1970			Women at risk 1968 1970[a]	Annual incidence
		MI	Coronary d ath	Total		
35 39	1	1	0	1	37418	0 03(0 05)
40 44	3	1	0	1	42263	0 07(0 09)
45 49	11	1	1		49594	0 22(0 26)
50 54	18	6		8	46131	0 39(0 56)

[a] Thos at ri k e ch year are totaled

Comparison between the inciden of MI in men and women

A comparison betw n th in idence of MI in men[30] and women r vealed that men predominat d in all ag groups (Fig 1) The incidence was about 8 time higher in men Non a idental unexpe ted sudden deaths outsid hospi tal w also much more frequent in men

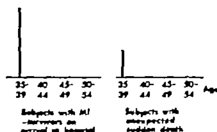


Fig 1 Sex ratio for MI (survivo s on arrival in hospital) and unex pe t d non a idental sudden d ath. Th ee year in id nce in Göt borg

DISCUSSION

The in idenc figur s in th pr s nt hapt were obtained by studying the incidenc of MI in the total g n ral population of subj t aged 54 or younge in Göteborg In Table II a compari on i made with th in iden found in a population sample of women in Framingham U S A [2] and in the total popu lation of women in Kristian und Norway[70] and H lsinki Finland[154] Th

Tabl II Incidenc of MI (sudden death not in lud d) p 1000 women pe y ar in Framingham[42] Kri tiansund[70] H lsinki[154] and Göt borg

Ag (y ar)	Incid n of MI per 1000 pe y r		
	Framingham[a]	Götebo g[b]	
30 44	0	0 05	
	Kristiansund	Göt bo g[b]	H lsinki
30 34	0	0	0
35 39	0	0 03	0
40 44	0	0 07	0 06
45 49	0	0 22	0 90
50 54	0 6	0 39	0 97

[a]Ag t ntry to the tudy Figu alculat d f om 4 yea inciden [b]Ag at th beginning of th yea Ag t on t of th ymptom

incid nce of MI in women seems to be similar in Göteborg Framingham and Kristiansund However it is to be noted that th women at risk were few in both Framingham and Kristiansund The incidence was higher in women aged 45 or older in Helsinki than in Göt borg The incidence was similarly higher in men in Helsinki [154] than in men in Göteborg[50] The number of sudd n d aths was also larger in Helsinki Göteborg and Helsinki have about the same num b r of inhabitants and th way of life s ems to be similar in th two cities The difference in the incidence of MI and sudden death between Göteborg and Helsinki is th refore surprising and cannot as yet be explained

In agreem nt with previous studies in other places [54 164 186] the incid nc of MI and sudden death was higher in men than in wom n in Göt borg

An important que tion is whether all new cases of MI in the ar a were r gistered. In th Göt borg area there is only one hospital for acute heart di seases which simplifies the registration of all hospitalized cases Almost all subj cts with MI in Göteborg are taken to hospital[61] The number of unre gistered cases with symptomatic MI was less than 10 % [I 50]

Th occurrenc of sil nt MI" in the area is not known. However none of the women participating in the population study in Göteborg[15] demonstrated ECG abnormalities suggestive of a previous MI (Minnesota Code 1 1 or 1:2[II]) This finding may suggest that silent MI is infrequent in women in Göteborg Although th occurrenc of silent MI is probably low it cannot be n glected. The incidence rates thus do not include individuals with non fatal silent MI MI resulting in sudden death however is almost always recognized as about 80 % of women who die from cardiovascular dis as outsid hospital in Göte borg are autopsied[90] El ven of 14 women (79 %) who had died suddenly out ide hospital in th present study w re autopsied If autopsy has not been p r formed sev r precordial pain preceding death strongly sugg sts an acute MI It has thus been report d that 20 of 23 patients who had sudden death pre ced d by central chest pain had a fresh coronary artery thrombosis and/or fresh MI on autopsy[116]

Roman numerals r f to the chapters of this monog aph Arabic numerals ref r to the referenc list pp 122 128

Chapter IV

CLINICAL CHARACTERISTICS OF YOUNG WOMEN WITH MYOCARDIAL INFARCTION

Call Bengtsson and Gösta Tibblin

A b t r a c t A hi tory of p evious myocardial infar tion and angina pectoris was more common in women with an acut atta k of myocardial infarction than in the g n ral population of women as w re symptom of angina pe tori and int rmittent claudication Chroni bronchitis gallstone di ea e and renal tone dis s were also ov r repr sented The women with myocardial in farction w re usually inactive wh n taken ill About one third of them om plained of breathl en s and/or disturban e of consciousn ss as ociat d with th atta k Most wom n with myocardial infarction had symptoms su h as nausea vomiting and cold per piration The infa ction area wa localiz d with about th sam frequency to th anterio and po terior wall of th heart In about half of the women the maximum registered t mper ture exc ded 38 0°C The primary mortality of th women who were ho pitali d wa rather low (9 %) Th ove all mortality among thos who w r survivors on arrival in hospital and those who died outside hospital during th first few hours or days wa 30 %

Myoca dial infar tion (MI) in women i ra e below th ag of 60 compared to men of th sam age[11,111] Thus there are few r ports on th clinical picture of MI in young and middle ag d women Previous report [13,19 165] have usually dealt with clinical ri s which cannot be onsider d as repr entative for the total numb of women with MI in a special g ographical area Some oth pr viou studi hav included wom n with oth r manifestation of ischa mic h art dis as (IHD) than MI[91 30 39]

Th pre ent report deals with th total number of MI in women born in 1913 o lat r who lived in th defined area of Göt borg Sweden during th years 1968 1970 Som haract risti and a de ription of clinical f tur s in these women ar given

MATERIAL

All subje ts with an acut ttack of MI who w e born in 1913 or later and who live in Göt borg have b en registered in th 1st of January 1968 at the Post MI Clinic[1,90] Death rtifi at s and utopsy report of pe ons born in 1913 or late and who lived in th sam rea have been tudi d in orde to in lude non h sp tali d ubj t dying from MI Th pre ent mat rial wa oll ted during th y a 1968 1970 On woman aged 23 with systemic lupus rythematosu was excluded s h r MI wa on d ed to be a omplication of h primary unde lying dis a[23] The eri compris d 47 women who were survivor on arrival in ho pital Four of th m di d befor being interviewed by membe of th Post MI Clini staff Another 10 women who w re not hospitali d fo heart di a di d suddenly from MI[1] The deaths of another

4 women who were not known to have a history of severe heart pain preceding death and who had coronary atherosclerosis but no fresh MI area on autopsy were classified as coronary deaths[1]

METHODS

In connection with th acute attack of MI the women w re interviewed in a standardised way by one of five physicians from the Post MI Clinic Angina p ctoris (AP) and intermittent claudication were defined as proposed by Rose[155] Chronic bronchitis was d fined as coughing and sputum in th morning and during th day or night for at least three months a year[123] A history of gall stones or renal stones was obtained by means of a questionnaire The examina tions during the hospitalisation period were performed in a standardised way in the medical wards Eight en women (38 %) were taken to the coronary care unit

Routin ly the rectal temp rature was recorded twice daily ECG and deter minations of SGOT w re performed on each of the first three days A leucocyte count was done during the first or se ond day Laboratory analys ECG re cording and X ray w re p rformed according to the routin m thods used at Sahlgr n s Hospital Göteborg SGOT was determined as Carmen units before July 1st 1970 and as U/l after this date Two Carmen units correspond roughly to one U/l up to th level of 100 Carm n units (50 U/l) Valu s ≥ 40 Carmen units or ≥ 17 U/l wer consider d as pathological X ray was per formed with the patient standing The roentgenological heart size was calculated from frontal and lat ral films[90]

S t a t i s t i c a l m t h o d s Th hypoth sis of diff renc s in fr quenci s betwe n groups was tested by m ans of the binomial distribution with a normal approximation

Further details of material and methods are given in Chapter I

RESULTS

A g e

The ag s of th women who were hospital ed for MI are shown in F g 1 Th youngest woman was 37 y a s old wh n she suffer d MI The ages of those who were la fi d a MI d ath and coronary death are shown in Tabl I

H i s t o r y o f c h e t p a n d a u t o p s y f i n d n g s i n t h e w o m e n w h o d d

Tabl I shows the pr val nc of severe heat pa n and autopsy findings n wom n who d d s dd nly nd who w not hospital s d for suspect d MI Tw lv of th s women d d out d ho p tal whil 2 w inst tutionalised for asons oth r tha h art d e (b a t a and strok r spe t v ly)

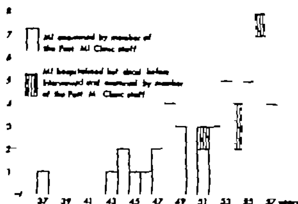


Fig 1 Age di tribution of wom n with MI in Göt borg during th years 1968 1970 born in 1913 o later (sur vivors on arrival in hospi tal)

Table I Age pr en of s vere chest pain pre eding death and autopsy findings in wom n who di d suddenly and unexpect dly and who had not been hospitalized becau of suspe t d MI

Subject No	Age (y ar)	Ch st pain	Autopsy	Fre h MI area on autop y	Sev r coronary athero l rosis on autopsy
Cla sifi d as MI[1]					
1	40	+	Yes	+	+
2	42	+[a]	Ye	+	+
3	48	+	No		
4	51	+	Ye		+
5	51	+	No		
6	51	+	Y	+	+
7	53	+	Y s	+	+
8	54	+	No		
9	54	+[a]	Y s	+	?
10	56		Y s	+	+
Cla sified a oronary death[1]					
1	48		Ye		+
2	54	+	Y s		+
3	55		Y		+
4	56		Ye		+

[a] Acut h rt failur p dominating symptom p eding death.

Table II History or prevalence of som diseases in 47 women with MI sur vivors on arrival in ho pital compared to a r ference group (578 participants in a population study of wom n in the ag strata 50 and 54)

	Women with MI %	Reference group %	Significance of diff r nce
History of MI	12 8	0	$p < 0$ 001
History of s vere ch st pain ≥ 30 minut s	23 4	0 5	$p < 0$ 001
Angina pectoris	46 8	3 3	$p < 0$ 001
Chest pain om time	70 2	6 4	$p < 0$ 001
Intermittent laudi ation	11 1[a]	1 7	$p < 0$ 001
Chronic b onchitis	20 5[b]	2 9	$p < 0$ 001
History sugg sting gallstone di ase	35 6[a]	21 8	$p < 0$ 05
History sugg sting r nal tone dis ase	13 6[b]	5 0	$p < 0$ 001

[a]Data from 2 women missing [b]Data from 3 women mi sing

Hi tory of pr viou di ase

In Table II the women with MI who w survivors on arrival in hospital, are compar d with regard to history or p valenc of om previous di ases with a repr sentative sample of women f om the same g neral population in th ag trata 50 and 54 [I,15] Th women of thi ref ence g oup had a m an age similar to that of the women with MI[I] Six of th women in th MI group (13 %) had a history of pr viou MI and almost half of th m eport d symptoms of AP Symptoms of intermittent claudi ation and hroni bronchitis and a history of gallstone and renal ston s w r also more common in th MI group than in the r f ence group (Table II) Information about history of MI and AP was obtained for 7 of 10 women who had di d f om a MI attack without being ho pitaliz d f r heart di ase Fiv of th 7 women had a history of AP whil non of th m had a history of MI

A t v ty wh n t k n ll

Informat on about a tivity when tak n ill wa obtain d from all th 47 sur vivo Thirty n (83 %) tat d that they w re at r t when taken ill on (2 %) that sh wa und mental t ss and s ven (15 %) that th y w e phy ically a t v ? on of th m wa und v physical tra n Valid inf rmation wa obt in d f only a f w of tho who di d out d ho pital

T m wh t k ll

Th tta k of MI oc u d f qu ntly during th day as during th ght

Information was obtained from all the 47 women Ninet n (40 %) suff ed th attack during th day 18 (38 %) during the night 6 women (13 %) in th morning and 2 women (4 %) in th vening whil 2 women with pain lasting for m re than 24 hours on a rival in hospital could not say wh n th pain began Valid information was obtain d conc rning 9 of those who di d outsid hospital or who w r in titution li ed for r asons other than su pect d MI The attack tarted during the day in 4 of them, in the vening in 2 and during th night in 3 The attack of survivors tend d to occur mo e oft n during th autumn and winter than during the pring and summer whil the death w re evenly distribut d during th y ar (Fig 2)



Fig 2 Seasonal varia tion of MI in women White columns MI survivo on a rival in hospital dark co lumn MI deaths triped column coronary d ath

Symptom onn t d with th acut attack

Information was obtain d only about those who w re survivors on arrival in ho pital

Pain Inf rmation wa obtained from 46 women Pain was noted in all of th m. In 9 women (20 %) th pain was localiz d to the ternum only n 2 wo men (4 %) to th st rnum nd n ck and/o jaw in 26 (57 %) to th t rnum and arm and/or should r and in 9 (20 %) to th st rnum a w ll as to th n ck and/or jaw and arm and/o hould rs Information about pain was n t obtained in one woman wh e main initial symptom wa sudd n los of con ciousn

Br thl ne Information wa obtain d from 45 women Of the 30 (67 %) had had no diff culty in bre thing whil 15 (33 %) complain d of shortn s of br th

Disturban of ons iou n ss Information wa obtain d from 43 women Thirty of the (70 %) had been fully con iou ll the time 8 (19 %) omplained of having had a f ling that verything g w dim for a moment and 5 (12 %) had be n un on ou for some time

Autonom n vous system di tu ban Information wa ob tained from 44 wom Nine of thes (20 %) did n t complain of ithe nausea vomiti g o old pe sp ration Four women (9 %) omplained of nausea 2 wo m n (5 %) vomit d 7 (16 %) n t d old pe piration and nother 22 women (50 %)

noted cold perspiration and at the same time ither felt nauseated or vomited

Signs noted during the period of hospitalization

Signs of heart failure Signs of left ventricular insufficiency were reported in 14 out of 47 women (30 %) either by physical examination or X ray or both Pulmonary ral s were not d in 9 women (19 %) Radiological signs of congestion w re noted in 9 (29 %) of 31 women who had had chest X rays at the time of the acute attack In one woman manifest pulmonary oedema occurred

Arrhythmia In 30 of 47 women (64 %) some kind of arrhythmia was re corded mostly supraventricular tachycardia and/or supraventricular extra systoli beats (11 subjects) and ventricular tachycardia and/or ventricular xtrasystolic beats (15 subj cts) Atrioventricular block was not d in 5 sub jects Ventricular fibrillation that could be regularized occurred in two women One of th m recover d and was able to l ave the hospital as a onvalesc nt Th oth r became unconscious in association with th attack of ventricular fi brillation and died a few days later

Body t mperature and laboratory data

Maximum not d values for rectal temp rature and some laboratory data are given in Tabl III Twenty six out of 47 women (55 %) had a maximum tempera ture ≥ 38 0°C Leu ocyte ≥ 10 000 wer found in 26 women (55 %) In all but 5 subj ct pathologi al SGOT valu s w r recorded

Tabl III Number of subje ts within different int rvals of r ctal temp rature and some laboratory values in women with MI (maximum not d values ar re port d)

T mperatur (°C)				
< 38 0	38 0 38 9	39 0 39 9		≥ 40 0
21	20	5		1
Leu ocyt s (pe mm³)				
< 10000	10000 14900	15000 19900		≥ 20000
1	14	9		3
SGOT (C rmen units)				
< 40	40 99	100 199	200 499	≥ 500
2	13	8	9	2
SGOT (U/l)				
< 20	20 48	50 99	100 250	≥ 250
3	5	3	2	0

Lo ali ation of the infarction are

As judged from the ECG th anterior wall wa aff ct d in 23 subjects and the posterior wall in 19 subj ct (Tabl IV) Q wav s w re record d in 32 sub j cts (68 %)

Tabl IV Localization of the infa ct on area a co ding to ECG

Localization	n	%
Anterior wall	15	32
Lat ral wall	2	4
Posterior wall + s ptum	6	13
Post rior + lateral wall	13	28
Anterio + lat ral wall	8	17
Un rtain localization	3	6
Total	47	100

H art volume

Ch t X ray was performed on 37 wom n befo e l aving th h spital The relative heart volume (ml/m^2 body surfac) was found to be < 400 which i regard d as a normal valu in 19 women (51 %) 400 499 in 12 (32 %) and ≥ 500 in 6 (16 %)

Out om of th a ut attack

Four of 47 women who w e ho pitali ed f MI (9 %) died during th period of ho pitalization In luding thos who died outsid ho pital or whil hospita lized fo as one oth r than su pected MI 14 of 57 women (25 %) di d during th acute attack If thos classifi d as oronary death ar included the primary m rtality i found to be 30 % (18 of 61 women)

DISCUSSION

Studie of young and middle ag d women with MI are few and usually per formed r tro pectively on el ct d s ri Th p sent eries omprise al most all women with MI during a defin d period of time [1] and th subje ts w re not examined retrospectively but in onn ction with the acut tta k A plans fo th investig tion existed befo e th subj ct f ll ill ther re few missing data among the survivor Th clinical hara t ristics of th women with MI in the p sent study are th refor onsidered to be rep esentativ for women surviving an a ut atta k of MI Limited inf rmation was obtain d

concerning th women who died outsid hospital

A hi tory of a pr vious attack of MI was common being recorded in 13 % of tho ho pitalized This is l ss than has be n r po t d arlier Thus Black man and Kologlu[19] r port d a pr vious history of MI in 33 % of wom n under 50 years of ag and Ball y and Beaven[13] in 2 % in a s ries of women wher no ag limit was fix d Howev th s studi s w re perform d on elect d hospital seri s

AP wa not d in almo t half of the wom n which i in agre ment with pre vious reports It wa al o common in those who di d from MI outside hospital Shoch t[165] report d a history of AP in 20 % of women with MI ag d 50 or younger and Blackman and Kologlu[19] in 40 % of women under 50 y a s of age A imilar pr val n e of AP (45 %) wa also not d in men with MI in Göteborg[201]

A history of oth r ch st pain not d fin d as AP wa r port d in another 11 wom n in th p nt s ri s Thi may w ll be in agr ment with the notion that AP is often atypi al in women[9] It i possibl that atypical chest pain may be a manif tation of IHD

It has also b en tat d that MI i oft n atypical in women[71] but this was not onfirm d in the pr s nt study Int rmittent laudication another mani f tation of art riosclerotic disea was also ove r p ent d in th pr sent MI g oup It must be born in mind that th p nt data were obtained in as ociation with an a ut attack of MI It is po sibl that th subject are mor awar of ch t pain tc and th r for report it mo oft n than those parti ipating in population tudy but thi annot xplain the gr at diff renc betw en th two group

Chroni bronch tis was common in th wom n with MI which may be x plain d by n over r p e entation of smok s in subj ct with ch onic bron h ti as w ll as n subj cts with MI[171] In agr ment with previou observa t ons on m n in Göt bo g[20] a history of gall ton or r nal ston was more ommon n th women with MI than in th referen group

The ut atta k usually occu red wh th wom n w r at r t and in th night a oft a n the daytim The risk of having MI whil und physical t n m d to b mall Simila obs rvation on MI urvival a d sudd n d ath ha be r port d f om H lsinki F nland[4] nd on medi ally unatt nd d d th f om Stockholm Sw d n[200]

C mpa t ly f w att k a m d to occu during th summ Th mat rial i too mall t llow lu on on rn ng s a onal variation but th t nd ncy found agr s th th t nd s in tudi of wom el ewh r[4 5 70]

Symptom h naus omiting and old per piration w re v ry common oc urri g h mo tha half of th wome

About thi d of th wom n ompl ined of b athl sn s and/o di tu ban of on or Sig of pulmonary ong t on n t d on phy ical or

X ray examination were common Th heart volum s emed to be increa d in ome of the wom n Most of the women had som kind of arrhythmia Supra ventricular or ventricular tachycardia and/or supraventricular or ventricular beat w re p dominant manif stations As most of th women had not be n taken to the coronary car unit minor episod of arrhythmia wer probably often not record d T mp rature and laboratory data usually d viated moderate ly from normal values The r sults are sentially simila to thos in a pre vious ho pital series [19]

The anterior and post rior wall of the heart s emed to be affe ted with the same fr qu ncy which is in agreement with autopsy findings in a study of men and women ≦ 45 year of age in Finland [120]

The mortality as ociated with the ho pitalization fo th acut attack was ather low in the pr sent material (9 % of those hospitaliz d) Altog th r 30 % of the women with MI o other coronary death died within th first few hours or day aft the ymptom appeared

Roman num r ls f r to th chapt rs of thi monog aph Arabi num rals ef r to th f renc list pp 122 1 8

Chapter V

BLOOD PRESSURE IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calle Bengtsson

Abstract Women with myocardial infarction had hypertension were on antihypertensive treatment and had a family history of hypertension significantly more often than women in the general population Th distribution of blood pr ssure valu s in women with angina pectoris did not diff r significantly from that in th g neral population of women Hypert nsive were significantly ov r represent d among women with ECG chang s suggestive of ischa mic heart dis ase This might be interpret d lthe as a sign that coronary ECG as defined is often a pr symptomatic manif station of ischa mic heart disease in hypertensiv s or that thes ECG changes are not specific for ischaemic heart disease

Several communications have r ported an a sociation between high blood pr ssure (BP) and ischa mic heart dis ase (IHD) in men [147 144 176] There ms also to be an association in women although the literature her is parse and controversial

Th purpo of the present hapter is to des ribe th arterial BP in a re pr s ntative populat on sample of women and in wom n with IHD Spe ial re f rence is gi en to the que tion whether women with manifestations of IHD diff r from women in the g n ral population

MATERIAL

Reference group A population study of women in Göt bo g was performed in 1968 1969[1] Women in th ag strata 38 46 50 54 and 60 w r examin d 1462 women altog ther (participat on rat 90 1 %) The total number of wome partı pating in the population study provid d th r feren group when ompari g parametri variabl s in this chapter When looking for diffe en es in frequ nc s of n n par metric variabl s between g oups of women w th IHD and women in the g neral population those aged 50 and 54 (n 378) provid d th r f ence group They had about th sam mean g as th women with IHD

Wom w th angina p ctoris Twenty nin women in th population study repo t d hı tory of angina pe toris (AP) as d fin d according to Rose [5]

Wom w th orona y ECG ECG changes implicating Minnesota Codes 1 1 2 4 1 5 1 2 (in th bsence of 3 1) 6 1 7 1[9 H] w r defin d as coronary ECG d re o d d in 23 women parti pating in the population study

Wome w th my rd al infa ct on All people in Göteborg born in 1913 o lat wh ha had an cut atta k of MI have been regist r d since the 1st of January 1968 t th Post MI Clinic n Göt borg [I] Forty s ven

women who we e urvivor on arrival in hospital we e regist red during the y ars 1968 1970 Of th se 4 women di d during th period of ho pitalization and one soon aft rwa d Forty two survivo s w r admitt d to clinical con trol 3 and 12 months aft r th MI atta k[1,51] Anoth 14 women who w r n t hospitaliz d for suspe ted MI died from a p obabl IHD attack during th period 12 out ide hospit l and 2 during a period of hospitalization fo asons oth r than susp ted MI[17] T n of th s wom n w re cla sifi d as MI de ths[1]

METHODS

BP measu ments in th population study Th parti ipants of th population study w examined according to a special chedule[15] Phy si al examination including determination of BP wa performed about half an hour after th i arrival at th pla of examination All th BP det rminations wer performed by th author The am manomet r wa u ed on a h occasion BP wa measu ed betw n 07 30 10 30 a m in fa ting subj ct in a qui t oom. The t chnique of BP d t rmination was that r commended by WHO[7] which had al o be n us d in the previous xamination of men in Göt bo g[107] After about 5 minut onversation with th woman who wa sitting omfort ably in a hair a 25 x 12 m cuff with nylon bookl t binding wa ppli d firmly and evenly to th right arm. The lower dg of the cuff wa about 2 cm above the ant cubital pa The cuff was quickly inflated to 200 mm Hg and the st th scope wa applied to th dge of th cuff Th cuff pr ssur was low red at a rat of about 2 mm Hg pe pul beat and the point at which the pulse beat wa first audibl wa cord d Diastolic p su was d fin d as the point of muffling of ound (phas 4) and the point of disappearan (phase 5) When the ystolic sound wa audibl immediat ly afte inflation th cuff pr ssure was allowed to fall to e o and a fr sh inflation to 250 mm Hg was performed Th BP was read to th nea t 2 mm Hg Reading w r pe formed in the seated po ition in all individual and in the supin and tanding positions (immediate ly aft r r ing) in all but th fi st 33 women tudi d

Det mination of BP in th wom n with MI BP was det min d 3 months and 1 y ar afte the attack of MI Th d t rminations w re mad in the morning and with th same t chniqu a d ribed above BP was det rmin d in the supine and standing po ition by on of five physician from th Po t MI Clini

Hi to y of a t ial hyp rt n ion All participants both thos ttending th population tudy and tho with MI wer interviewed conc rning hi tory of art rial hypert n ion Tho on ntihypertensive treatment we re o ded s parat ly Information on tho who had died wa obtained f om ords and medi o l gal report

Family history of a t rial hyp t n i Family hi tory of

arterial hypertension was obtained in the same way

Electrocardiographic examination Th ECGs were taken in a standardized way[I] and read according to the Minnesota Code[u] with its Scan dinavian modification[9]

Statistical methods

Conventional statistical methods were used to calculate mean values and standard deviation (S D) The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approxi mation

Furth r d tails of mat ial and m thod ar given in Chapter I

RESULTS

BP in a population sample of women

The m an values of BP at diff rent ages are given in Table I In th e ated and upin positions both pha e 4 and phase 5 of the diastolic BP are given and in the standing position phase 4 only All individuals in whom BP had been determined are included even those on antihypertensive tr atment at th time of th examination De entil limits of BP in th seated position are given in Fig 1 Distributions are given in Figs 2 3 The number of women on antihypertensiv treatment is also shown in thes figures and together with the type of treatm nt in Tabl II Saluretl diuretics w the antihypertensive ag nts most commonly u d.

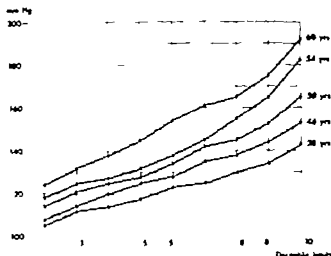


Fig 1 Decentile limits of sy tolic BP in the seated position The Study of Wo men in Göteborg 1968 1969

Table I M ans and S D of BP (mm Hg) in th s ated supin and tanding positions
The Study of Women in Göteborg 1968 1969

Ag	Seat d			Supin			Standing		
	n	Mean	S D	n	Mean	S D	n	Mean	S D
	Systoli BP								
38	372	123	11 5	362	124	14 5	362	128	16 1
46	431	131	19 5	420	133	21 2	420	132	20 5
50	397	138	21 8	385	140	22 7	384	137	22 5
54	180	143	24 1	180	145	23 9	179	141	22 5
60	81	154	27 5	81	158	27 3	81	152	27 2
	Diastoli BP phas 4								
38	372	81	9 2	362	80	10 7	362	88	9 3
46	431	85	10 9	420	84	10 2	420	90	10 6
50	397	88	10 6	385	87	11 4	384	93	11 4
54	180	89	12 1	180	89	11 5	179	95	12 3
60	81	92	12 3	81	93	12 3	81	96	13 5
	Dia toli BP pha e 5								
38	372	79	9 2	362	78	9 6			
46	431	82	10 0	419	82	10 2			
50	379	86	10 6	384	85	10 7			
54	180	87	12 0	180	86	11 5			
60	81	89	12 4	81	90	12 4			

Tabl II Number on antihypert nsive t tment
Th Study of Women in Göteborg 1968 1969

Ag	Salur ti diu ti		Oth than aluretic		Salur ti s and oth		Total	
	n	%	n	%	n	%	n	%
38	3	0 8	1	0 3	0	0	4	1 1
46	5	1 2	4	0 9	5	1 2	14	3 2
50	13	3 3	1	0 3	4	1 0	18	4
54	9	5 0	1	0 6	9	5 0	19	10 6
60	11	13 6	0	0	2	2 5	13	16 0

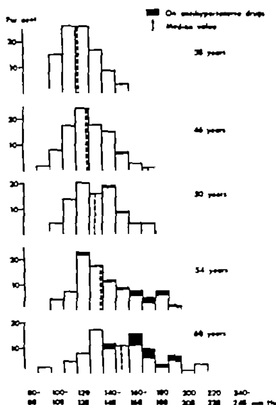


Fig 2 Distribution of systolic BP in the seated position The Study of Women in Göteborg 1968 1969

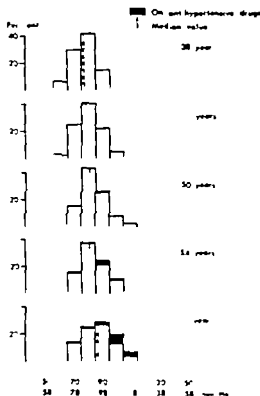


Fig 3 *Distribution of diastolic* BP in th a ated position Th Study of Women in Göt bo g 1968 1969

BP in non attenders

Information about th BP on at least on occasion du ing th previous three y ars was obtained in 95 (74 %) of the 128 r fu I by studying reco ds f om inpatient and outpati nt clinics and by int rviewing th women T e tment for hypert n ion was as common in th refu rs f om whom information had been obtain d as in th att nde s ex ept fo tho women ag d 54 in whom a great r pe entag of refu rs than att nd w iving treatm nt (Table III) Two of th refu rs had be n told that th ir BP wa high but did not know the valu and 37 of them had be n told that th ir BP wa normal but did not know the values Fifty ix wom n (44 %) had be n told th valu of th ir BP o information wa obtain d f om the re o d Th m an systolic BP was omewhat high r in these fu ers than in th att nde s (Tabl IV)

Table III Antihyp rten ive treatment in r fuse s from whom information had be n obtained (n 95) as c mpared to th attend (n 1462)

Ag (yea)	R fu r n	R fu r %	Attend r %
38	0	0	1 1
46	1	2 9	3 2
50	2	7 1	4 5
54	6	33 3	10 6
60	2	14 3	16 0

Tabl IV Systolic BP (mm Hg) in fu rs in whom th BP had been corded during the previous 3 years

Ag (y a)	n	M an	S D
38	13	131	15 3
46	15	139	16 0
50	16	140	24 4
54	8	171	20 8
60	5	155	21 2

BP in wom n with AP and oronary ECG in th population ampl

Angina p toris In Tabl V women with AP ar plac d a cording to d entile limit of systolic BP found in diff rent ag in th population sampl Th wa a light tr nd towards a high r BP in women with AP Thu 17 wo men with AP w found in th upper and 12 in th low 5 de entil of syst li BP Th ove r pr sentation of women in th uppe 5 d ntil wa not statistically ignificant Th r sult of th statisti al analysi i the sam if the woman on antihypertensive treatment who i found in the third d ntil i taken to th upper 5 d centil s

Coronary ECG Th prepond ran of valu above the median (Tabl V) wa mo ma k d in th wom n with oronary ECG than in thos with AP and wa tati tically signifi ant ($p < 0.01$) El ven of 23 women with o onary ECG w on ntihypertensive therapy and an th r two w on diureti s fo oth r ons

Table V Women with AP (n 29) and with coronary ECG (n 23) placed in d c ntiles of systolic BP as found in th population study (Fig 1) irrespec tive of wh th r on antihypertensive drugs or not (tho e on antihypertensive treatment also separately in brackets)

Decentile of systolic BP in th seated position									
1	2	3	4	5	6	7	8	9	10
				Angina pectoris					
3	2	3	2	2	4	3	3	2	5
		(1)				(1)	(1)	(1)	(1)
		12 (1)					17 (4)		
				Coronary ECG					
1	1	1	2	0	3	1	3	3	8
			(1)		(1)		(3)	(1)	(5)
		5 (1)					18 (10)		

BP in wom n with MI

A history of art ial hypert n ion (l vat d BP in conne tion with p gnancy only not included) wa found in 22 of 47 women with MI who w e survivors on a rival in hospital compa ed to 71 of 578 women (12 %) in th r f onc group Th diff r nc wa stat stically ignificant ($p < 0.001$) Sixt n of 47 women (34 %) with MI we on antihypert nsive drugs ompared to 37 of 578 in the r ference group (6 %) th diff r nc being tat sti ally significant ($p < 0.001$) Information was obtained onc rning 7 women who d ed f om an atta k of MI but who w r not ho pitalis d for suspected MI Five of the 7 wom n (71 %) had a history of hypert nsion

Th systolic and diastol c BP in th wom n 3 and 12 months ft th MI ar shown in Figs 4 5 Aft r the infar t on th r was no definite diff r nc in BP betw n th s women and tho of th population s mpl How ver 10 of 38 (26 %) w r on ant hypertens ve drugs 3 months aft r the MI compared to 37 of 578 (6 %) n th f n group Nin teen of 40 (48 %) w r on antihyper tens drug 1 y ar ft r th MI

F m ly hi to y of t l hyp t o

A o ding to th format on g n rt l hype t n ion w r port d in on o both pa t n 11 of 29 wom n w th AP (38 %) n 9 of 23 wom n w th oronary ECG (39 %) d n of 41 MI vivo s (63 %) ompa d to 208 of 548 wom n (38 %) in th popul t on mpl The MI g oup diff red statisti ally signifi tly f om the g n l popul t on of wome in thi re pe t ($p < 0.001$)

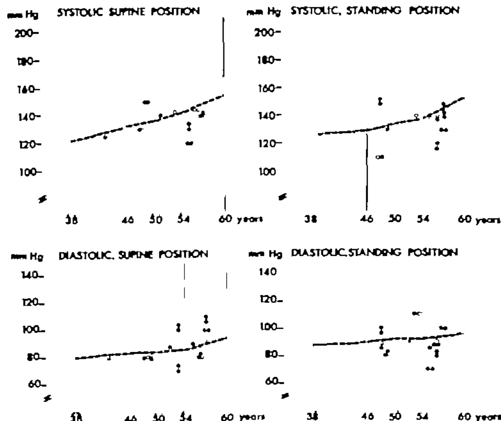


Fig 4 BP in the supin (n 38 data on 4 survivo mis ing) and tanding (n 37 data on 5 urvivor mi ing) po tion in women with MI 3 months aft r th infar tion The dott d lines ombin the median value of th population sampl of women in the age group 38 46 50 54 and 60 Fill d symbol denot tho on antihype t nsiv treatm nt

while th wom n with AP and th women with o onary ECG did not Art rial hyperten ion wa report d in on or both pa ents of 4 of 6 women who di d without being ho p tali ed for MI and in whom a family history had be n obtain d

DISCUSSION

The BP valu found in th pre ent study w in ag ment with th results of pr viou studi of w men in Scandinavia [34, 7] but omewhat low than in Framingham U S A [72] nd in T cums h U S A [89] They we similar to th found in men in Göt borg [82]

The attend n rat wa high in the p nt population study of women being 90 % of tho drawn f om the gen ral population [5] Thi means that the data on the BP obtained from th population study are liabl for th total population in th se ag group This i furthe t engthen d by the info mation obtained about the on ttend r About th sam proportion of w men among th

Tabl V Women with AP (n 29) and with coronary ECG (n 23) placed in decentiles of systolic BP as found in th population study (Fig 1) irrespec tiv of whether on antihypertensive drugs or not (those on antihypertensive treatment al o separately in brackets)

Decentile of systolic BP in the seated po ition									
1	2	3	4	5	6	7	8	9	10
Angina pectoris									
3	2	3	2	2	4	3	3	2	5
		(1)				(1)	(1)	(1)	(1)
		12 (1)					17 (4)		
Co onary ECG									
1	1	1	2	0	3	1	3	3	8
			(1)		(1)		(3)	(1)	(5)
		5 (1)					18 (10)		

BP in wom n with MI

A history of art l l hyp rten ion (levated BP in connection with p gnancy only not in luded) was found in 22 of 47 women with MI who we e survivo s on arrival in hospital compa ed to 71 of 578 wom n (12 %) in th f r nce group Th diffe n e wa statistically ignifi ant ($p < 0\ 001$) Sixteen of 47 women (34 %) with MI w r on antihypert nsive drugs compa d to 37 of 578 in the ref renc group (6 %) th diff renc being statistically significant ($p < 0\ 001$) Information was obtain d con rning 7 wom n who di d from an attack of MI but who w r not hospitali d for susp ct d MI F ve of th se 7 women (71 %) had a history of hypert n ion

Th sy tol and diastolic BP in th wom n 3 and 12 months aft r th MI a hown in Fig 4 5 Aft r the infar tion th wa no d finite diff c in BP betw n th s women and thos of th population sampl How v r 10 of 38 (6 %) w e on nt hyperten i e drugs 3 months after th MI compared to 37 of 578 (6 %) i th r f renc group. I n t en of 40 (48 %) wer on antihyper tensiv drug 1 y ar ft r th MI

F m l h to y of t al hyp rt n on

A o d g to th info mat on g v n art l hypert n ion was r port d in on or both pa t 11 of 29 wom n w th AP (38 %) n 9 of 23 wom w th o ona y ECG (39) d 2 of 41 MI urvi or (63 %) compa d to 208 of 545 w me 3 %) th popul t on sampl Th MI g o p diff d statisti lly g f antl f n th gen l pop l t on of wom n in this r pe t ($p < 0\ 001$

clusions from BP d t rminations after th MI must be drawn with aution The impre sion that th r is no diffe nc in BP betw n wom n with MI and thos in th gene al popul tion (Figs 4 5) may be o t fo women who att nd Post MI clinic but this do s not n ce sarily m an that th ir BP wa th sam before th infar tion Wom n with MI w al o mor oft n on antihypertensive drugs 3 and 12 months aft r th nfarct on than wom n in the g neral population.

Data concerning p xistent hypert n on a p obably mor valid for om parison Women with MI we significantly mo e oft n on antihyperten ive tr atment b fo th infarct on than wom n n the g n ral popul tion and mo e oft n had a history of hype t n ion Th p val c of hypert n ion in th pre sent eri s of women wa imila to that ported in women with MI (survivo) and sudd n d ath in H lsinki Finland[154]

Subj ct with MI had h t pain befo the infa ction mo e often than women in th gen al population[II] This might hav mad th m vi it a doctor and hav their BP ch ck d mo fr quently whi h may induc bias in th int pr tation of the results How v it will probably not xplain th diff enc found

Information c nc rning family hi tory m to b mo unbi s d and a th familial facto i onsid ed to be important fo hyperten ion[24] it wa tudi d in th p nt mat ial A ording to th information volunt red, the women with MI had hyperten ve parent gnificantly mo oft n than th wo men in th r f ren group

Th present study does not how a d finit a soc tion betw n AP and hyper tension in women although th sult f om F amingham[02] indi at thi

A larg majority of th women with o onary ECG in th p sent tudy w r on antihypert n v t tment o had high BP Only few women with o onary ECG w found to have oth manif tation of IHD[II] It i pos ible that th o onary ECG a defin d n th p ent tudy i not pe ifi f r IHD but rath r di tingui he subj cts with hypert nsion[I] Howeve a th seems to b a conn tion betw n arterial hypert n ion and ath o l osi it i also po ibl that th oronary ECG p symptomatic manif tation of IHD Th finding that a family hi tory of hyp rt n i n wa not m r ommon in the women with on ry ECG than in th gen ral population of women i difficult to xplain

A p o pe tive study with a f llow up of a ro ct onally studied popula tion would be th id al way to obtain info mation about th imp rtan of fa to s such a i d BP Th sult of th T um h and Framingham p o spective studi are of int rest in thi ontext Thus Epst in[55] port d f om the T cum h tudy that th incid n of IHD in middl ag d w men up to th ag of 60 wa about th sam regardl of th valu of thei initial diast li BP whil ft th g of 60 tho with a BP in th uppe quintil had an inci den of IHD ght time that in th low quintil In Framingham[102] the in i

dence of IHD was higher in women with a BP > 160/95 and even in women with BP > 140/90 than in women with a BP < 140/90 (women aged 30 62 when they ntered the study) This tendency was also found when th incidenc of MI and AP were studied separately However the numbers of women with IHD were small in th se prospective studied Th low frequency of MI in middle aged women makes such studi s difficult if a number of women with MI large enough for valid statistical analyses is required [I]

Roman nume als f to th hapt r of this mon g aph Ar bi num ral r f r to the r f ren li t pp 12 128

SERUM CHOLESTEROL AND SERUM TRIGLYCERIDE LEVELS IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calle Bengtsson, Per Björntorp and Elisabeth Tibblin

Abstract. The serum cholesterol levels of women who had suffered myocardial infarction were not higher than in the general population of women, nor the serum cholesterol levels of women with angina pectoris or ECG changes suggestive of ischaemic heart disease. The serum triglyceride values were significantly higher in women who had had myocardial infarction and in women with ECG changes suggestive of ischaemic heart disease. There was no significant overrepresentation of high serum triglyceride values in women with angina pectoris.

Prospective and retrospective studies have demonstrated a statistical association between high serum cholesterol values and ischaemic heart disease (IHD) in men.[71,94,167,168,199] Some investigators have also found elevated cholesterol values in women with IHD.[5,130] The literature on serum triglycerides is not as extensive but does indicate an association between high triglyceride and IHD in men.[13,71,94,184]

The levels of cholesterol and triglycerides in women aged 38–60 as found in a population sample of women in Göteborg are described in detail elsewhere and related to previous studies.[180] The purpose of the present paper is to compare the cholesterol and triglyceride values of women with angina pectoris (AP), women with ECG changes suggestive of IHD ("coronary ECG") and women who had had myocardial infarction (MI) with those of women in the population sample in order to find out whether there are differences between women with these manifestations of IHD and women in the general population.

MATERIAL

Reference group. A population study of women in Göteborg, Sweden, was performed in 1968–1969.[1,15] Women in the age strata 38, 46, 50, 54 and 60 totaling 1462 women were examined. These women constitute the reference group.

Women with AP. Twenty-nine women in the population sample reported symptoms of AP defined according to Rose.[55]

Women with coronary ECG. ECG changes defined as coronary ECG were recorded in 23 women participating in the population study.

Women with MI. All people in Göteborg born 1913 or later who have had an acute attack of MI have been registered since the 1st of January 1968 at the Post-MI Clinic in Göteborg.[1,30] Forty-... women who survived on arrival in hospital were registered during the years 1968–1970. Of these

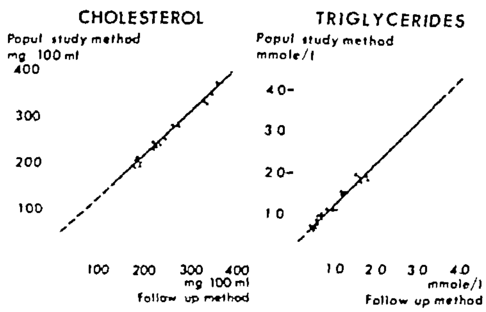
4 women died during the period of ho pitalization and one soon afterwards Forty two survivors w re admitted to clinical controls 3 and 12 months after the MI attack [5?] Another 14 women died from a probable IHD attack outside hospital or while hospitalized for reasons oth r than suspected MI during the period T n of th se women were classifi d as MI death [2]

METHODS

Blood ampling of the women participating in the population study was per formed after overnight fa ting as d scribed pr viously [15] Serum cholesterol and triglyc rid oncentrations of these wom n w re determined according to th m thods d scribed by Levine and Zak [2] and Loftlan [115] respectively The analytical steps aft r the xtraction of lipids wer automated Th chol sterol and trigly c ride valu s of the wom n with MI w r d termin d according to th same m thods dur g th period of hospitalizat on xcept in 10 pati nts whos chol sterol valu s were d termin d according to Franey and Amador [()] with minor modif cat ons Th valu s obtained with the latter m thod clos ly agre d with thos obta n d with th previous method

Th chol t rol and triglycerid det rminations at th 3 month and 12 month controls aft th MI w arr d out according to th m thods d scribed by Cramér and I ak son and Carlson [12] r p t ly

Th orr l t ons between v lues obtain d with th methods used in the popu l tion study d at th linical controls are hown in Fig 1



F g 1 F l t p t F l at o a d tr gly r d (n 26) d t rm at or o h m thod d th f llow p t dy of th women wh had ad I d th pop l t on t dy pe t ly

5

Reg e sion equations wer for chol st rol y 0 918x + 32 5 (mg/100 ml) 0 96 and fo triglyc rid s y 1 025x + 0 249 (mmole/l) r 0 97

Th m thodological rror was calculated on duplicat analys s as $\sqrt{\frac{\Sigma d^2}{2 N}}$ whe e d is th diff rence b tw n duplicat and N th number of ampl The erro of th method was th n found to be 4 4 mg/100 ml for chol terol and 0 05 mmol /l fo triglyc rid s in the population study d t rminations and 6 6 mg/100 ml and 0 04 mmol /l respectiv ly in th follow up d t rminations

Stati ti al method

Conventional tatisti al m thod w re us d fo calculation of mean values standard d viation (S D) and orrelation oeffi ient (r) The hypothe i of diffe ences in fr qu ncie between group was t t d by means of th binomial distribution with a no mal approximation

Furth r d tail of mat rial and methods are given in Chapter I

RESULTS

Se um chole t rol and s rum trigly ride l vels in middl ag d wom n

A detail d report on rum chol te ol and rum triglyc rid levels in the p ent population sampl is given el ewhe e[140] M ans S D and d c ntil limits a shown in Tabl I and in Fig 2 3

Tabl I Serum chol st rol and s rum trigly id lev l
Th Study of Wom n in Göt borg 1968 1969

Ag (year)	n	M an	S D
Chol st ol (mg/100 ml)			
38	371	241	34 8
46	429	260	53 6
50	398	276	41 9
54	178	284	41 7
60	81	283	35 0
Triglyc rid s (mm l /l)			
38	371	1 09	0 44
46	430	1 20	0 65
50	398	1 6	0 58
54	178	1 39	0 69
60	81	1 34	0 62

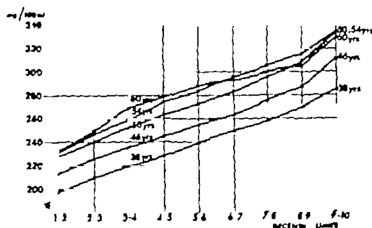


Fig 2 Dec ntile limit of serum cholest rol levels The Study of Wo men in Göteborg 1968 1969

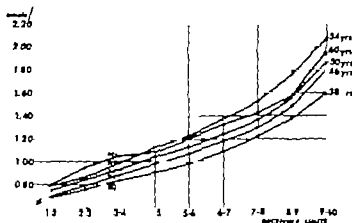


Fig 3 Dec ntile limits of serum *triglyc ride levels* Th Study of Wo men in Göt borg 1968 1969

Angina p to s In F g 4 th wom with AP ar placed in dec ntiles of chol st rol and triglyc rid valu s according to th value in th *population* study Th re w s no stat t ally gnifi ant over rep es ntation of wom n with AP who had valu in th upp r 5 de nt les (above the med an) fo trigly rid s Sev nt n wom n had valu s above nd 12 below th m dian No diff renc was found f r ch lest rol (14 wom n who had AP w re above nd 15 w e below th m dia) Only a f w women h d co exi tent high chol t ol and trigly rid valu s Nin women w found to hav val s abov th m dian fo both h l t rol d tr gly erid whil 7 women wer found to hav valu s below the media fo both Thre of the wom n with AP w on lip d r du ing *drug* (lof brat) t the t m *of th exami tion One of th m had cholest rol* and triglyc rid valu abo e th median and anoth who had previously had high hol st ol alu wa found to be below the m dian for both Th third woman had a hol t ol v lu below the med an and a triglyc id valu bove th median If th r ult n the women on lipid r du ing drugs w disca d d and th ir valu w on id d to be bo th m dian fo chol t rol and

AP

CHOLESTEROL (Decentiles) × TRIGLYCERIDES (Decentiles)

	1	2	3	4	5	6	7	8	9	10
1	1	(1)		1		2				
2										
3										1
4							1			
5			1	2	1				1	
6	1						1			
7			1		1	1	1			
8	1									1
9			2	(1)			1			1
10		1					1		1	(1)

"Coronary ECG"

CHOLESTEROL (Dec n-tiles) × TRIGLYCERIDES (Decen-tile)

	1	2	3	4	5	6	7	8	9	10
1										
2	1									
3										
4			1		1		1			
5			2	1						
6			1			1			1	
7									1	
8	2	1								1
9		1				2			1	1
10				1	1					1

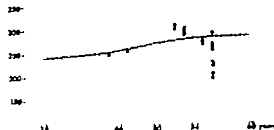
Fig 4 Women with AP and co onary ECG 1 sifi d according to de entil s of s rum hol st rol and rum triglyc rid value as found in the population tudy (tho ir 1 d on lipid r ducing drug)

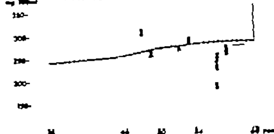
triglyc rid s irr pe tive of th valu s found th stati tical analy is would still have given th am sults

Coronary ECG In Fig 4 the women with oronary ECG ar plac d in d centil of chol te ol and triglycerid value acc ding to the valu in the popul tion tudy Ten of them had valu s abov and 13 below th m dian fo chol t rol whil 16 women had value abov and 7 below the median of tri glyc ride Th over p ntation of trigly ride values above the m dian wa stati ti lly gnificant ($p < 0.05$) Non of the women with o onary ECG wa on lipid redu ing drug

S um hol t ol and s rum t igly id l vel in wom n w th MI

Individual h l st l and triglyc rid valu of women with MI at th time of the acut attack and at 3 and 12 months lat ar shown in Fig 5 Adju tments w re mad in the figures in o d to omp nsat fo the diff en du to diff nt m thod f rum lipid stimation t th follow up ontrols and in the population tudy Th numbe of ubject who had value above and below the medians of th popul t on ampl (ft dju tment fo th diff rent m thod of rum lipid d termination) i summariz d in Table II Th re was a ignificant o pre nt tion of women w th trigly d value above th medi n t th tim of th acut atta k and at th f llow up ontrol aft rwa ds The hol t ol valu of th women with MI w re no h ghe than n the gen ral popula tion of wom n Fou of th women w th MI w re on a l pid du ing g nt (lofib t) t th tim of th ir MI 2 w men aft 3 month nd 8 wome aft





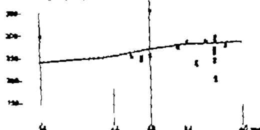


Fig 5 Individual rum chol sterol and s rum triglyce id valu in women bo pitalized for MI at the tim of th acute attack and 3 and 12 months lat r Fill d symbols denote women on lipid redu ing drug The lin combine the median values of th f male population aged 38 46 50 54 and 60 a found in the populat on study befor ( ) and aft r ( ) adju tment for th diff rent method of rum lipid d termination n the populat on study and at th 3 month and 12 month ontrol

12 months If th valu of the women who were on a lipid reducing drug were regarded a being bove th m dian irr spectiv of th levels found, th r sults f om th statisti al analys s would till have be n th same

Table II Numb r of women hospitaliz d fo MI who had valu s abov and below th median fo serum hol sterol and serum triglyceride l vels in the popula tion sample (number on lipid reducing drug is also given s parat ly in brack ts)

	Above m dian	B low median	Total	Significan e of differen
	Acut			
Ch l st rol	15 (3)	23 (1)	38	NS
Triglyc rides	29 (3)	8 (1)	37	p < 0 001
	Afte 3 month [a]			
Cholsterol	14 (1)	23 (1)	37	NS
Triglyc rid s	34 (2)	2 (0)	36	p < 0 001
	Aft r 12 months[a]			
Chol st rol	19 (5)	20 (3)	39	NS
Triglyc rid s	36 (7)	3 (1)	39	p < 0 001

NS No tatisti al gnifi an

[a] Adjusted to comp nsat fo th diff rent m thods of rum lipid d t rmina tion at the 3 month and 12 month ontrol and in th population tudy

A high chol ste ol valu was oft n not a companied by a high trigly rid value in th ame individual For instan of 20 women who had a triglyc rid value in the uppe 2 d ntil t th 3 month ontrol 8 were above and 12 below the median valu of h l terol at the sam ontrol

Information w s obtain d bout the rum h l st ol valu of 6 women who di d from MI out d ho pital Two of them were known to have had valu > 400 mg/100 ml

DISCUSSION

Prospect v tudi of men have shown that a high hol st ol valu is a o iated with an in d i k of MI a vi w d among othe by Staml [6] Ca lson and Lind t dt[34] and Simb rg[67] Pr pecti tudi on triglyc rid ar f w so fa but g vid n that l d trigly d ar a ociat d with an inc a d ri k of MI in men[33, 34] How v r t ms from the tudi on men in Göt borg[206] that rum trigly r d v lu giv no extra inf rmation for predicting th ri k in men when erum hol t l valu ar vailabl

Due to th low d n of MI young and middl g d women prospe tive studi s are more diff cult to perform n women ithe a very larg numbe or very long follow p period ry In th p o pecti tudi pe formed in Framingham nd T ums h[5] no distinction wa mad betw en th

various manif stations of IHD in women due to th low incidenc rat In th Framingham Study high cholesterol se med to be a risk factor for IHD in women aged 30 49 but not in wom n ag d 50 59 while triglycerid measured by mean of an ultracentrifuge gave a better prediction of IHD than cholesterol l vels in thes older wom n[51] In the T cums h Study[55] no a sociation was found between high cholest rol valu s and IHD in women

Case control studies like the present one can probably give a somewhat false picture as th MI per se may in some way reduce the lipid values Thus chol esterol and oth r lipids are known to fall aft r a MI and the valu s do not ri e until several we k later[67 147] In a prospective study of men cholesterol and triglyceride values when m asur d 3 months after MI were simila to the pre infarction valu fo the sam individuals[202] which mean that rum lipids measured 3 months aft r a MI are not much bias d by th MI attack

In the pre nt study serum lipids w re examined at th time of the a ut attack and aft r 3 and 12 months when pr infa ction values we thus expect ed Although no low fat diet was prescribed initially th subje t themselves may have chang d their dietary customs Le en[110] not d a decr ase in rum cholesterol of about 55 mg/100 ml in m n who had had MI and who were pre scribed di tary restrictions and a decr as of about 10 mg/100 ml in another g oup of men with MI to whom no instructions had be n given When abnor mally high follow up values wer noted in the pr sent study di tary advic and/or lipid r ducing drugs w re pres ribed aft r the 3 month control These possibiliti s of influ nce on the serum lipids must be kept in mind but a e pro bably of minor importance The different methods for serum lipid d t rmina tion used in the pr sent study are anoth r pos ible sourc of error although efforts wer made to compensate for these diff rences

In the pres nt tudy th wom n with MI or other manifestation of IHD were not found to hav high hol sterol valu than th women in the g neral popu lation Con lusions must be drawn with caution be ause of the fa to discu s d above However a s rum lipid 3 months aft r th MI are probably n t b as ed by the MI attack[202] t ms unlik ly that th cholesterol valu s of th wo men with MI we highe than in the gen al population even before th r attacks Trigly er d w re gnif c ntly higher in the wom n with MI and in the women with coronary ECG and tended to be highe in th women with AP than in th general population

In gen ral th hol terol and trigly eride valu s of the women with IHD were within the no mal rang or on or th other showed an levation Only a few had co sis tent high chol st ol and trigly eride valu s

Chol sterol ha in previous studi been found to be higher in women with MI[5] and in women with IHD[30] than in normal It was al o higher in hyper tensiv women with IHD than in hypertens women fre from IHD[35] The

r sults from thos studi s thu mostly disagr e with the r sult from anoth r cas control study[147] in whi h no significant diff r nces were found betw en women with MI below the age of 60 and th ir control from the pro pe tive studi in Framingham[93] and T cum h[55] and from the pr nt tudy Howev r ome of the ri s w r v ry small[45,135] and none of the previous cas cont ol studies discuss d used randomiz d subjects from th population a control Mul ahy et al [130] noted that the diff ren e in s rum chol t rol betw n women with IHD and controls was particula ly p ominent in the young st ag group (women aged 35 44) which agre s with th results from Framingham[93] There are too few women with MI below th ge of 45 in the pre nt study to allow parate conclusions onc rning this ag range

The r sults of p vious tudi hav shown that triglycerides tend d to be high r[5] or w significantly high r[1 7] in women with MI than in control and w high r in hypert n ive women with IHD than in hyp rtensiv women fre from IHD[135] Th sults gr senti lly with thos from Framing ham[93] and with th p nt re ult

R man num al f to th hapt r of thu monog ph Arabi numeral f r to th f en li t pp 122 128

Chapt r VII

SMOKING HABITS IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Call Bengtsson

Abstract Smoking was more common in younger than in older women in the general population as found from a population study of women ag d 38 60 Almost half of the women aged 38 were smok rs as compared to about 20 % of thos ag d 60 Th re was no over repres ntation of smokers in wom n who had angina pectoris or ECG changes suggestive of ischa mi h art disea e Women who had an acut attack of myocardial infarction wer significantly mor often smokers than women in the g neral population In addition iga rette consumption wa ignificantly higher among femal smok rs who had MI than among female smokers in th g neral population

Smoking is con id red to be a ociated with ischaemic heart di ease (IHD)[10] Most previous information has been ba ed upon studi s of m n with IHD In th pres nt chapter the smoking habits of women with manifestation of IHD ar ompared with thos of women in the gen ral population

MATERIAL

Refer n e group From a population sample of women[I 15] tho of the ag 50 and 54 (578 wom n) onstituted th ref ren e group They had about th ame mean ag as the wom n with IHD[I] Th moking habits of th total sampl (1462 women) ar also d scribed

Wom n with ang na pectori Tw nty nine women participating in the population study po t d symptoms of angina pectori (AP) as defin d a cording to Ros[155]

Wom n w th coronary ECG ECG hang d fined as o onary ECG[I] we re ord d in 23 women parti pating in th population tudy

Women with MI All peopl in Göt borg born in 1913 or later who hav had an acut attack of myocardial infarct on (MI) hav been regi ter d sinc th 1 t of January 1968[L,50] Forty seven women who wer survivors on a rival in hospital w re reg t red du ing the y 1968 1970 Anoth r 14 women di d from a probabl IHD attack outside ho pital or while hospita li ed fo a ons other than uspected MI[IV] 10 of whom w re classifi d as d aths from MI[I]

METHODS

Int v w onc n ng mok ng hab ts

Th women pa t c pating n th population study were int rviewed about th ir smoking hab t uch a type of smoking (c gar tt ciga s pipe) amount of daily onsumpt on and fo how long they had be n smoking Th y w re also

asked whether they inhaled or not. Non-smokers were asked whether they had smoked earlier in life and ex-smokers were asked when they had begun and when they had stopped smoking. Women with MI were interviewed in the same way at the time of the acute attack. The daily consumption was recorded as 1-14, 15-24 or ≥ 25 cigarettes in the MI group. Subjects who smoked ≥ 1 cigarette per day were defined as smokers.

Statistical method

The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation.

Further details of material and methods are given in Chapter I.

RESULTS

Smoking habits in the population sample

Smoking was much more common in the younger than in the older women (Table I). Almost half of the women aged 38 were smokers compared to about 20 % of the women aged 60. The percentage of ex-smokers was about 10 % in the youngest and oldest women and about 6 % in the middle three age strata. Only a small number of the ex-smokers had stopped smoking during the previous year. More than 90 % of the smokers were inhalers. Eleven women (about 1 % of the women in the age group 38-54, none in the age group 60) smoked cigars and/or a pipe. Seven of them also smoked cigarettes. Table II shows the daily consumption of cigarettes among the cigarette smokers. Most of them smoked 5-14 cigarettes a day. The median daily cigarette consumption among the smokers was 10 cigarettes in all age groups.

Table I. Smokers, ex-smokers and non-smokers (%).
The Study of Women in Göteborg 1968-1969.

Category	Age (years)				
	38	46	50	54	60
	n 372	n 431	n 398	n 180	n 81
Smokers, inhalers	44.6	41.5	34.9	34.9	17.3
Smokers, non-inhalers	1.9	2.8	2.3	3.9	2.5
Ex-smokers who stopped smoking last year	1.1	0.9	0.3	0.6	3.7
Ex-smokers who have not smoked during the last year	6.5	[illegible]8	3.3	4.4	2.5
Ex-smokers who have not smoked during the last 15 y	3.0	2.3	2.8	0.6	3.7
Non-smokers (ex-smokers not included)	42.7	49.7	56.5	55.6	70.4

Table II Cigarette smoking wom n (% of the total number of cigar tt smok rs) classifi d according to daily ciga rette consumption and age
Th Study of Women in Göt borg 1968 1969

Cigar tt s/day	Ag (years)				
	38	46	50	54	60
	n 173	n 189	n 147	n 69	n 16
1 4	10	18	15	20	19
5 9	0	29	28	9	19
10 14	36	28	39	33	37
15 19	18	15	9	11	19
20 24	13	8	8	6	6
≥ 25	1	2	1	0	0

Smoking habits in the women with AP and coronary ECG in the population sampl

Angina pectoris Eight of 29 women with AP (28 %) were smok rs and 6 (21 %) ex smok rs Fift en women (52 %) had never been smokers The per c ntag of wom n who had never been smok rs is about the same a in the re f rence group Th percentage of mok rs in th AP group is low and is low r than that of any group in th population sample except for the ag group 60 Four of th 6 ex smok rs had stopped smoking more than 8 years before th population study

Coronary ECG Three of 23 women with oronary ECG (13 %) were smoke s and 2 (9 %) ex smok r Smoking wa l ss ommon in th wom n with coronary ECG than in any of the age groups in the population sample

Smoking habits in the wom n with MI

Information about smoking hab ts was obtained from 46 women with MI Thirty seven women (80 %) w e smokers 1 (2 %) was an ex smok and 8 (17 %) w e non smok rs (Tabl III) Smoking was much more common in th women who suffered MI tha in th referen e group The women with MI who smoked were also significantly mo e often heavy smokers than the femal smok r participating n th population study None of th women with MI mok d ciga s o a pipe Confirm d information wa obtained for only 3 of the women who di d f om MI out de hospital On of thes women wa a smoker on an ex smoker nd th third woman had n ve smok d

Table III Smoking habits and cigar tt consumption in women hospitalized for MI compared to a r f ren e group of women aged 50 and 54 participating in th population study

Category	Women with MI n	%	Ref rence group n	%	Significance of differen e
Smokers	37/46[a]	80	218/578	38	$p < 0.001$
Ex moker	1/46[a]	2	35/578	6	NS
Non mok rs (ex smoker not included)	8/46[a]	17	325/578	56	$p < 0.001$
Smoke s and ex m ker	38/46[a]	83	253/578	44	$p < 0.001$
1 14 cig/day[b]	1/35[c]	60	177/216[d]	82	$p < 0.001$
≥ 15 cig/day[b]	14/35[c]	40	39/216[d]	18	$p < 0.001$
Inhalers[b]	30/34[e]	88	202/218	93	NS
Non inhal s[b]	4/34[e]	12	16/218	7	NS

NS No statisti al significan e
[a]Information from one woman m sing [b]Among those moking [c]Information from 2 mokers missing [d]Two women who smoked xclusively cigars or a a pip not in lud d. Information f om 3 smoker missing

DISCUSSION

A larg number of communications have onfirm d the a sociation betwe n tobacco smoking and IHD[107 1 2 175 206] Autopsy findings hav rev al d a orrelation betw en th d gree of oronary ath o clerosis and th amount of cigatte smoking in men Th onnection between moking nd IHD seems to be st ong fo MI and mo dubious fo AP[44 61 184] Nl otin ha be n discu sed a a possible causative g nt g by K rschbaum t al[101] while A trup t al[10] have focused th inter t on th pos ible ath rogenic ff ct of carbon monoxide in smok rs Howev t has be n much d bated wheth r th high in id nce of MI in smok s is aus d by moking per or if there exi t some othe common causative fa to o factor in smok and subjects with MI g anxi ty[79] or a sp ial behavioural patt rn[1, 65,156] R sult of twin studies although bas d on limited data do not onfirm th the ry of a dir t conn ction[64 13,117]

The debate on th rôle of smoking as a ri k f ctor fo MI ha be n focu d on men a they h v MI mor often than w men Howeve an incre s d mortality f om IHD in smok has been reported both in men and women[80] In th F amingham Study MI and fatal heart att k we mo common in women who mok d than n thos who did ot but th in iden of AP wa milar[36] In a study n New York (th H I P tudy) th incidence of MI was

signifi antly high r in wom n who smok d than in tho who did not but the incidence of AP was almo t the sam[162] Th data from th s studies agree with th obs rvation from th p esent study in Göteborg that smokers w re strikingly over repr s nted among th women with MI but not in the women with AP

Several of the women with AP w re ex smok rs Th y had usually stopped smoking s v al years p eviou to the population tudy which m ans that most of th m had not stopp d smoking as a cons quence of chest pain

Th re wa no over rep e entation of moke s in th women with "coronary ECG in th p sent study On th contrary mokers tend d rath r to be und r repr sented in this group This may pos ibly be anoth r indi ation that the coronary ECG in women as defined i not mainly a manif station of IHD[I II]

Smoking of iga ett s was found to be about as ommon in the pr s nt study of wom n in Göt borg as in pr vious studie of women in Framingham U S A[96] B rg n Norway[44] and Glostrup Denma k[73] In Glostrup a furthe 18 % of wo men ag d 50 mok d cigars and/o a pipe compa ed to 1 % of th wom n in Göt borg of th ame age

Smoking has be n hown to b ontinuously on th increas in women[29] and is more common in th younge t than in the old st ag group as noted p viously[22 48] and in th present study The diff renc in smoking habit b tween young and old women giv s rise to an int sting qu stion Will MI be mo common in wom n when th young women of today who are heavy smoker a h what may be call d nfar tion ag ?

Roman num al r f to th hapt rs of thi monograph Arabic nume al r fe to th l en li t pp 122 128

DIABETES MELLITUS CARBOHYDRATE TOLERANCE AND EARLY INSULIN RESPONSE TO AN INTRAVENOUS GLUCOSE INJECTION IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calle Bengtsson Göran Blohmé and Johan Waldenström

Ab tract Th prevalence of manife t diab t s m llitus was significantly higher in women with myocardial infarction than in wom n in the general population This was not the ca for wom n with angina pectori or ECG chang s suggestive of ischaemic heart disea Ther w no diff renc in fasting blood glu ose or fasting serum insulin valu s betw n non di betic women who had angina pectori or who had had myoca dial infar tion and non di betic women in the general population Int avenous gluco injection did not r veal significant diff rences in glucos tol ran e or mea valu s of ea ly insulin ponse Howev r ther was an over repr sentation of women with a v ry low arly insulin re pons in th women who had had myocardial infarction

Manif st diab tes mellitus as w ll as l ss d gr of impai d glucos tolerance have been found to be associat d with i h emi heart diseas (IHD)[14 144 168 193] A d cre d and delayed glucos timulated arly insulin r sponse is on id red to be a ommon featu in subject with diab tes[77 105] The insulin r spons has u ually be n found to be eith r normal o incr a ed in subj cts with IHD [18 132 3 65] Th purpo of th p nt chapt r i to bring forth furth r data on thi matt r Women with myoc rdial infar tion (MI) and women in th gen ral population in Göt bo g ompa d with r gard to th prevalen of manif t diabet toleran to an ntrav nou (I v) glucose inje tion and early insulin spon to such an inj ction Som supplementary data are given con erning wom n with angina pe toris (AP) and wom n with ECG hanges suggestiv of scha mi h art di as (co onary ECG)

MATERIAL

R ference g oup A population tudy of women in Göt borg Sw den wa perform d in 1968 1969 Wom n n the ag trata 38 46 50 54 and 60 were examin d in all 1462 women [2, 5] Except fo mall b sampl th women ag d 50 w admitt d to ubsequent studi of the tol ran to i v gluco inj t on and th arly n ulin pons [20 21] (355 women pa ti ipation rat 85 3 %) Th m an g f th women wa t the time of th i v glu o t l anc t t (IVGTT) 50 9 ye s Two women with p viou ly known manif t di bet s t ated only with bohydrat re tri tion and on woman with pr vi ou ly unknown fa ting hype glyc mia w tudi d, whil two women n in ulin w ex lud d from th tudy Sevent n (4 8 %) of th women who were admitt d to the IVGTT had ontin ou ly be n on lureti dia ti du ing

th pe iod before the test

Wh n comparing th prevalence of manif t diabet th women ag d 50 and 54 in the population tudy totaling 578 women were chosen as a ference group as they had about the am mean age as th women with MI[I] A compari son of fasting venou blood glucose levels gluco e tol rance fasting insulin l v ls and early insulin respon was made betw en th women with MI and the 50 year old women participating in the population study IVGTT had be n carried out only in the 50 year old women in th population sample

Wom n with AP and "coronary ECG in th population sampl Twenty nine women in the population ampl had AP as d fined according to Rose[155] Twenty thr women had ECG change d fin d as coro nary ECG [I] The p eval n e of manifest diabetes and fasting blood suga values w re studied on all subjects with AP and coronary ECG IVGTT was perform d only on tho aged 50 in th population sample which m ans that 11 of ̣29 women with AP and 3 of 23 women with coronary ECG we e examin d by means of this test Th latt r group of 3 women is consid red too small to in lude in the p esentation of esults

Wom n with MI All peopl in Göteborg born in 1913 or lat r who hav had an acute attack of MI hav been r gist ed ince the 1st of January 1968 Forty seven women who were survivors on arr val in hospital were r gister ed during th year 1968 1970[1,50] Anoth r 14 women di d from a probabl IHD atta k outsid hospital or while hospitall d for r a on other than suspe ted MI[II] 10 of whom were las ified as MI deaths th others as coronary d aths [I]

Thirty ix of th women with MI wer admitt d to studi s of glucose tol ran and early insulin respons on average 16 3 months (range 3 36 months) aft r their MI attack They we th n ag d 45 58 (m an ag 54 1 years) One of th se women had manif t diabet Sh was treated with fenformin at th tim of th MI attack but had been on d etary strictions for two years when th IVGTT was perf rm d Fiv oth r women with manif t diabet s treated with insulin or oral hypoglyc mic ag nts wer not tud d furthe Seventeen (47 %) of the women with MI who were admitt d to th IVGTT had b n on saluretics during the period befo th IVGTT

METHODS

Informat on a to th preval nce of manif st di b tes wa obt in d from int rvi w Th women wer ons d r d to have manif st diabetes if before th population study o th attack of MI they had be n p cribed dietary r strictions nd/o oth tr atment for di bet All of th m had had glu o uria and elevat d fa ting blood glu ose valu On woman n th population mpl and on woman n the MI g oup with pr viously unknown diabetes had marked fa ting hypergly emia and glu osuria nd we on id d to have manif st

diabetes Th woman with MI gav a history of g at thir t for a long time befo the MI attack and ha sin the attack been tr at d with insulin

A fa ting v nou blood glucos valu of all participant in the population tudy was det rmin d by means of a ferric cyanid reduction m thod[82] adapted for autoanalyzer by T chni on N 26 Th methodological rror ($\sqrt{\frac{\Sigma d^2}{2N}}$ d diff r nces betwe n duplicate N = number of samples) was 3 0 mg/100 ml

The wom n who w admitt d to the IVGTT were all ambulatory and came to th laboratory in the morning after 12 hours fasting Salur ti were with drawn for 1 6 days and moking was not allow d during the last 12 hou befor th test Glu os 0 5 g/kg body w ight in a 50 % solution wa inj cted intra v nously during 2 5 3 0 minut s Z ro time was et at th tart of the inje tion. Immediat ly before th injection and at 4 6 and 8 minut venous blood sample w re taken fo blood glucos and erum insulin det rminations Betw en 25 and 60 minut venou blood amples w tak n very 5 minutes fo glucose d t minations Blood gluco was analysed within 2 3 hours S rum fo th in ulin determination was tored at 20°C in small aliquot for later analys s

In the spe imens withdrawn bef re and during the IVGTT glucos wa d ter mined in venou blood with glu os oxida using a commercially availabl reag nt (Glox® AB Kabi Stockholm) and with glycin buff r d pe chloric a id as p ot in precipitating ag nt[11] Th methodological rror wa 1 6 mg/100 ml The tol ran to i v gluco inj tion was expre d as a K valu (% d clin per minute) obtained from th slope of total blood glu o on a logarithmic s al from v nou blood glu os values betwe n 25 and 60 minut a originally d ribed by Hamilton and St in[79] The best fit of th traight line wa det r mined by minimizing the sum of square of the devi tion of th data point f om the lin Blood gluco valu s 10 mg/100 ml o l ab ve the fa ting l vel w re x lud d becau valu app oximating the fa ting l vel often deviat f om lin arity Serum in ulin wa determin d with a doubl antibody method orig nally de ribed by Hale nd Randl[75] u ing a omme cial radi immun a say kit (Th Radioch mi al C ntr Am ham U K) The tandard curv wa on tru t d u ing human nsulin whi h wa d live d togethe with the kit. Th rum was ncubated during 6 18 hour t +4°C A omparative tudy show d that inc ng th ond incubation to 40 hour r sult d in about 20 % highe valu Nothing was gain d in pr i ion Th methodological rror al cul ted from dupl at d t rminations was 1 4 mU/l at the 20 mU/l level and 3 mU/l at th 55 mU/l l vel A control ontaining 17 mU/l wa n lys d each day The day to day variat n ($\frac{S\ D \times 100}{mean}$) wa found to be 11 %

R l tive body w ght was expre s d a % of d l w ight[1]

Details of the IVGTT a p nt d parat ly[20 21]

Statistical methods

Conventional statistical methods were used for calculation of mean valu standard deviation (S D) and correlation coefficient (r) Significance of differ nces betw n mean values was stimated by Student s t to t As the distribution curve of K valu fa ting serum insulin values and erum in sulin values at 4 6 and 8 minutes were skewed[20 21] but the log values normally distributed the t st was appli d to the m ans of log values for th variabl s The distribution curve was also skewed for the values of rum insulin increas at 8 minutes while the log values w re normally distribut d As ome values wer negative a non parametric t st (Mann Whitney s U t st)[166] was applied for thi variable The hypothesis of differences in f equenci s between groups wa t ted by m ans of th binomial distribution with a normal approximation

Furth r detail of material and methods a given in Chapter I

RESULTS

Prevalence of manifest diabete mellitu

The population sampl Table I show the preval nc of manif st diabetes in the population sampl It was found that 0 8 % of the total number of women studi d had manif st diabete Among the women in the 50 and 54 years age group 6 women (1 0 %) had manif t diabet s

Information was obtain d by interview or by studying inpatient and outpatient e o ds from 119 wom n who fus d to participate in th population tudy e from 93 % of the r fus rs None of them had manif st diabet s

Table I Manifest diabetes prevalenc and mode of tr atment in wom n hosp talized be aus of MI and in a population sample of women

Age (years)	Subj cts at risk	Di t	Tabl ts	Insulin	P viously unknown	Total	
	n	n	n	n	n	n	%
W omen with MI							
43 57	47	1	2	3	1	7	14 9
Women in th populat on sampl							
38	372	0	0	1	0	1	0 3
46	431	0	0	0	0	0	0
50	398	2	0	2	1	5	1 3
54	180	0	1	0	0	1	0 6
60	81	3	2	0	0	5	6 2
Total	1462	5	3	3	1	12	0 8

Wom n with AP No of th women with AP had manif st diabet s

Wom n with oronary ECG One of 23 women with co onary ECG had manif st diabetes

Wom n with MI S ven of 47 women ho pitaliz d for MI (15 %) had manif st diabet This may be ompar d to six of 578 of the women ag d 50 and 54 who wer participating in the population study (1 0 %) Th difference is stati tically signifi ant ($p < 0.001$) Thr diabetic women with MI were on insulin t th tim of their MI and two w r on oral hypoglycemic ag nt On woman on diet and on previously untreat d woman w r given insulin at the time of the MI atta k Ex pt for one in ulin tr ated woman ag d 37 th age rang of th diabeti wom n with MI (45 54 years) was similar to the rest of th MI g oup (43 57 year Tabl I)

Two of 10 MI d aths outsid ho pital or whil hospitaliz d for r asons other than MI and one out of anoth r 4 d ath cla ified a o onary deaths had man f t diabet s Thu in total 10 of 61 wom n (16 %) who w re ho pitalized b us of MI or di d from a probable IHD tta k outside hospital or while hospitali d for r a ons oth than MI had diabet

Four women hospit li d for MI died during th p iod of hospitalization Two of them had diabet If th y a includ d th primary mortality was found to be 50 % in 10 diabeti women and 25 % in 51 non diabetic women among thos 61 women who w re hospitali d for MI or di d from a probabl IHD tta k without being ho pitalized for MI Th diff nce in primary mortality between diabeti and non diabetic women i not statistically significant

F ting blood gluco e

M an fasting venou blood glu os was imilar in th groups of wom n with AP coronary ECG and in the r f rence g oup of women ag d 50 when me sured at th popul tion tudy (78 4 75 2 and 75 mg/100 ml S D 14 4 16 2 and 16 7 mg/100 ml r pectively) It wa also similar in the MI g oup and in the f r n group (Tabl II the pe imen withdrawn when performing the IVGTT) Women on salu tics did not diff from thos who we not on iuretics

Glucose tol n e

Mean K valu s of the women who had had MI and of the women in the efe renc group did not diff gnificantly (Tabl II) In Table III th women are ub divided ac ording to th i K valu s T bl III al o includ 50 y a old women with AP Low K val (< 1 00) tend d to be ove p ented in the women with MI and in th women with AP compa d to the feren group but the diff enc were not significant The we e no diff rences between women who took and those who did not tak salur tics ith in the IHD groups or in

Table II Fasting v nous blood glucose values K valu s values for fa ting serum insulin serum insulin 4 6 and 8 minutes after i v glucos injection s rum insulin increase and body w ight in 36 women who had had MI and in a ref rence group of 355 50 year old women

	Wom n with pr vious MI			R ference group		
	M an	S D	Range	M an	S D	Range
Fasting blood glu ose[a] (mg/100 ml)	71 0	12 9	52 109	70 2	8 0	55 105
K value (%/min)	1 64	0 84	0 55 4 74	1 87	1 10	0 49 9 86
Fa ting serum insulin (mU/l)	15 1	9 1	4 42	14 0	5 3	3 47
Serum insulin 4 min (mU/l)	83 3	53 7	12 ₄10	86 2	47 0	12 390
S rum insulin 6 min (mU/l)	69 6	45 0	16 230	72 1	45 7	12 495
Serum insulin 8 min. (mU/l)	57 1	37 8	11 10	60 6	37 4	14 380
Serum insulin incre se at 8 min (mU/l)	41 9	36 6	3 176	46 6	35 6	11 333
Body weight (kg)	66 1	9 8	50 8 90 8	67 2	11 4	44 7 112 4
Relative weight (%)	108 2	16 1	82 144	108 9	16 9	76 174
Log K value	0 163	0 215		0 218	0 205	
Log fasting serum insulin	1 116	0 235		1 116	0 159	

[a]One woman in the MI group and 3 women in the r ference group w th manif st diabetes are excluded

the reference group

Fast ng e um neul n

Th fasting serum in ulin valu s of the women who had had MI did not diff r significantly from tho n th reference group (Table II) no did tho of the 50 year old wome with AP (mean 16 3 mU/l S D 6 1 mU/l if one woman with a v ry high value 47 mU/l s ex luded) The corr lation oeffi nt (r) between fasting insulin and lat v body w ight was found to be 0 31 in the r f rence group and 0 33 in the MI group

Table III Di tribution of K values in 35[a] women with previous MI ag d 45 58 in 11 50 year old wom n with AP and in a refer nc group of 35.[b] 50 y ar old women

K value	Women with MI		Women with AP		R ferenc group	
	n	%	n	%	n	%
< 0 90	4	11	0	0	21	6
0 90 0 99	3	9	2	18	18	5
1 00 1 09	2	6	1	9	22	6
≥ 1 10	26	74	8	73	291	83

[a]On woman with manifest diabet s excluded. [b]Three women with manif st diabet s exclud d.

The fasting in ulin valu w re significantly higher in th women on alur tics than in thos who were not on alur tic in the MI group (m an 19.2 and 11.5 mU/l, respe tively $p < 0\ 01$) They tended to be high r in thos on saluretics than in thos who w not on saluretic in the referenc group (17 4 and 13 8 mU/l respe tively $p < 0\ 10$) The mean relative body weight was highe in th refe ren group among women on saluretics (118 % as compared to 108 % among thos who wer not on saluretic $p < 0\ 05$) In the MI group there was no signi ficant diff ren e (108 % and 109 % re pectively) However 4 women on salu r tic with a r lative weight ≥ 130 % and high fa ting insulin valu s of 25 35 mU/l mainly ontribut to th high r mean value of fasting s rum insulin in the women on aluretl s in the MI g oup

S rum insulin r spon

M an rum in ulin l vels measured 4 6 and 8 minut s after th beginning of th i v gluco e inj ction in the women with MI and in th reference group ar shown in Tabl II No ignifi ant differences in mean valu wer found. No did th valu for rum insulin increas at 8 minute differ significantly betw en th groups Howev 9 of 35 non diabeti women (26 %) in the MI group had an insulin in as at 8 minut within the lowe t decentile of the r f renc group (≤ 16 mU/l) Thi ove r p s ntation of women with a very low e rly insulin r pon in the MI g oup wa statistically significant ($p < 0\ 01$ two-tail d t st)

The corr lation coeffi ient ( ) betwe n K valu and in ulin incr a e at 8 minut s was found to be 0 40 in th efe en group and 0 42 in th MI group Non diabetic women who had both a low K value (< 1 00) and a v ry low insulin inc se aft r 8 minut (≤ 16 mU/l) w re ove repre ented in the MI group (14 % a compared to 4 % in the ref ren g oup $p < 0\ 01$ two tailed t t) whil women with a low insulin increas aft r 8 minut but a K val ≥ 1 00

were mor similarly distribut d in th non diabetic MI group and in th refe r nce group (11 % and 6 % r spectively) No differences were found in body weight or relative body weight between the MI group and the referen group (Table II) and th r wa no difference in the glucos do given as this was proportional to the body w ight Women on saluretics did not diff from those who wer not on salur tics

The valu for serum insulin increase at 8 minutes in the 50 year old wo m n with AP did not diff r significantly from those in th r ference group They were very high in 2 women (333 and 169 mU/l) one of whom also had a v ry high fasting serum insulin value The others had valu similar to those found in the reference group (mean 41 4 mU/l S D 15 2 mU/l)

DISCUSSION

The prevalence of manifest diabetes in wom n aged 50 54 was 1 0 % in th present population sample which is in agreement with previous reports[26 52 69] Du to th high participation rate and the information concerning diabet s being availabl for such a larg number of non participants the information on th prevalence in th population studied is consid red to be valid

There are too few women with angina pectori in the present population study to allow valid con lusion about this group Th fact that none of the wo men with angina pe toris had manifest diabet does not indi ate an association betw en angina pectori and diabetes in women Thi agrees with th obs rva tion from the Framingham Study[95] that AP was only slightly more common in women with diabet s than in non diabetic women

The pr valenc of manif t di betes in the women with MI wa significantly higher than in women in th gen ral population This is in agre ment with previou r ports[17 28 60 82 83 124 93] Garcia t al[64] reported from Framingham that IHD is espe ially frequent in diabetic women on insulin but a high ate of IHD in subj ts who hav mild diabet s of late onset h s also been report d[175] Death from IHD wa found to be mor common in wom n with diabetes than in non diabeti women in th Framingham Study[96] A similar tendency was s en in the p e nt study Domenge t al[3] tated from autopsy finding that dia bet is the mo t important singl atherogenic factor

D cr a d tol ra to o al glu ose ing tion has been considered to be a ommon featur n ubject with IHD as reviewed by Wahlberg[93]

An abnormal t l nc to v glu ose inj ction was not ignificantly mor common in non di betic women who had had MI than in the refer nc group in the present tudy This agrees w th the r sults of most previous studi s which have been carri d out ex lus vely or pr dominantly on m n with IHD[30,118,132,140,151,154]

In a study carri d out by Wahlberg[93] abnormal or bo d rlin K values were significantly more common in women who had had MI than in a female control

g oup Th women who had had a MI had a mean ag of 66 y ars and w re on average 11 year older than their control Low K values we more common in older than in younger subj cts in the MI group (men and women) but not in th ontrol group (men and women) The wom n who had had MI in that ri were on averag 12 year old r than in th pr e nt study It is thus possibl that an impaired tol ranc to i v glucos inj ction is a mor common f ature in older subj t who have had MI and the dis epant results in diff r nt stu die might be explain d in thi way The time betwe n th MI attack and th IVGTT may be another fa tor of importan Most subj cts in th study pre nted by Wahlberg[193] wer te ted 3 6 w ks aft r their MI R testing later on did not reveal any significant hang in glu os t l nce In another tudy[148] impaired tol ance to i v glucos injection was common immediat ly aft r an acute attack of MI (1 23 day) but normalization was usually not d aft r 6 12 months

Fa ting insulin has been found to be imila in subje ts with MI a in con trols[14 6 132,149] o high r[149] In the present study of women ranges and m an of fasting rum insulin valu were similar in th MI and in the ref ren g oup In ag ment with previou observations[13] obe ity wa correlat d to high fasting in ulin value in th pre nt study Th higher fasting insulin valu s in women who bef re th IVGTT had been on saluretic wer probably explain d by a high r d gr of obe ity in the wom n

Women who took and women who did not tak s lu tics w r studi d s para t ly s previous tudi have ugg t d a pos ible as oci tion betwe n impair d glu os tol ran and saluretic[27 106] and almo t half of the non diabetic wo men with a previous MI took s luretics compa d to only about 5 % of th non diabetic wom n in th ref r nce group With the ex eption of fa ting insulin valu s no diff n w found betwe n th wh took and thos who did not take salu eti s for th variabl s studi d with the IVGTT

Abnormally high in ulin spons aft o al glu o e loading ha be n re port d in s ri of predominantly mal subj ts with IHD[118 132 149 189] Aft i v glu ose inj tion to ubj cts with MI th rly insulin spon has be n found to be normal[78] In th pr ent tudy the rang of a ly insulin valu s wa wid both in th MI g oup and in the r fer n e group The m n valu s at 4 6 and 8 minut s did not diff ignificantly between the group

Th turnov r of insulin in pe iph ral blood is apid[33 179] Th insulin in c a at 8 minut s whi h w u d a a m a u of the a ly in ulin spons in th present study doe n t th f re fl t th nti insulin respons Thi insulin r spon an be lculated app oximat ly f om the insulin valu s during th nitial pha of th t st[33, 79] How v th e is a high c rr lation (0 99) betwe n th insulin incr s at 8 minut and the enti rly insu lin spons du ing the fi st 8 minute c lculat d in thi way[21]

The values for early insulin increase at 8 minutes did not diff r significantly between women with MI and women in the population in the present study How ver women with no or very low early insulin increase wer over represented in the MI group As d creased and delay d arly insulin re pons is consid red to characterize not only manifest diabetes and lesser degrees of impaired glucos toleranc but also th so call d pr diabetic state[17] the findings might indicate an association betwe n the prediabetic state and IHD

Roman numerals fe to th hapters of this monograph Arabi umerals refer to th referen list pp 122 128

Chapter IX

NUMBER OF PREGNANCIES, USE OF ORAL CONTRACEPTIVES AND MENOPAUSAL AGE IN WOMEN WITH ISCHAEMIC HEART DISEASE COMPARED TO A POPULATION SAMPLE OF WOMEN

Calle Bengtsson, Göran Rybo and Hans Westerberg

Abstract. The number of childbirths had been significantly greater in women with angina pectoris or ECG changes suggestive of ischaemic heart disease than in women of the general population. A similar tendency was found for women with myocardial infarction. Spontaneous and/or legal abortions had been significantly more common in women with myocardial infarction. None of the women with myocardial infarction, angina pectoris or ECG changes suggestive of ischaemic heart disease had ever used oral contraceptives. Eight women who had a myocardial infarct before the age of 48 were all still menstruating when they had the infarct. A retrospective comparison revealed that significantly more women who had suffered myocardial infarction were postmenopausal at the ages of 45 and 50 than women in the general population. Menopause at the age of 50 or earlier had also taken place significantly more often in women with angina pectoris and in women with ECG changes suggestive of ischaemic heart disease. Postmenopausal 50 year old women had higher serum cholesterol and serum triglyceride values than 50 year old women still menstruating but blood pressure was lower than in the latter group of women. These differences in serum lipids and blood pressure between premenopausal and postmenopausal women were statistically significant.

The fact that myocardial infarction (MI) is rarer and occurs at a later age in women than in men has usually been explained by the hypothesis that women are in some way protected from MI by ovarian hormones[170]. It has also been stated that women with early menopause are more prone to MI than women in whom the menopause occurs later in life. The rôle of ovarian hormones in the development of MI has also been discussed in connection with pregnancy and the use of oral contraceptives.

The purpose of the present chapter is to compare women with manifestations of ischaemic heart disease (IHD) to the general population of women in a defined geographical area with regard to number of pregnancies, use of oral contraceptives and age at the menopause.

MATERIAL

Reference group. A population study of women in Göteborg, Sweden was performed in 1968-1969[1,13]. Women in the age strata 38, 46, 50, 54 and 60 were examined, 1462 women in all. Those of the ages 50 and 54 (578 women) constituted the reference group in the present chapter. They had about the same mean age as the women with IHD[1].

Women with angina pectoris. Twenty nine women participating in the population study were found to have angina pectoris (AP) as defined according to Rose[155].

on the oth r women In view of th ir age they probably did not use oral contra ceptives (one of th m wa 42 years old and all others ≥ 48 y ars old)

Premenopausal and postm nopausal women

Fig 1 pr sent th women who had MI by age and menopausal stat All wo m n who were 47 years of ag or younger wer still menstruating at the time they had their MI However women with MI usually had menopause at a young r ag than women in th efer nc group (Table III) A r trospective compari son revealed that a significantly larger number of women w re postmenopausal at the ages of 45 and 50 in the MI group than among women in the gen ral popu lation Similarly ignificantly more women with AP and wom n with coronary ECG were po tmenopausal at th ag of 50 Information was obtain d for 4 wo m n who died from MI outside ho pital Th ir menopause occurred at 43 47 50 and 51 year r spectiv ly

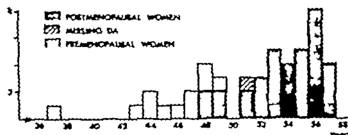


Fig 1 Women with MI survivors on arrival in hospital lassified according to wh th r menstruating or not

Tabl III Number of ho pitalis d women with MI and number of women with AP and coronary ECG who had r ach d the menopause at the ag of 40 45 and 50 re pe tively compar d to a r f renc group of women ag d 50 and 54 parti pating in th population tudy (% in bra kets)

	Women with MI	Women with AP	Women with coronary ECG	Reference group
M nopaus at the ag of 40 or earli	4/47 (9)	2/28 (7)	2/22 (9)	23/578 (4)
Menopau e at the ag of 45 or arli r	10/46* (2)	5/28 (18)	5/22 (23)	67/578 (12)
M nopau at th age of 50 or arli r	28/37*** (76)	14/22* (64)	12/17* (71)	280/578 (48)

* $p < 0.05$ *** $p < 0.001$ s gnif antly diff r nt from women in th g neral population.

Blood pressu and se um lipids in pr m nopausal and postmenopausal wom n

Th 50 y ar old women in th population sampl compri ed about the ame number of premenopausal and postmenopausal women In Tabl IV a compari son is made between thes two g oups with regard to blood pre sure and serum lipids which ar usually consid r d as main risk facto s for IHD It is seen that th systolic blood pre sure wa low r in th postmenopau al wom n while serum chol st ol and erum trigly rides w re rai d in the women The diff r nces wer statistically ignificant

Table IV Mean, tandard deviation (S D) and standard rror of the mean (S E) of art rial blood pr sure s rum h l t ol and serum trigly rid in premenopaus l and postmenopausal 50 y ar old women

	Premenopausal wom n (n 185)			Po tmenopausal women (n 148)			Signifi canc of difference
	M an	S D	S E	M an	S D	S E	
Systolic blood pressur (mm Hg)	140	22 4	1 64	135	21 4	1 76	p < 0 05
Diast li blood p sure (mm Hg)	88	10 5	0 77	88	10 9	0 90	NS
Serum chol terol (mg/100 ml)	266	36 4	2 68	286	46 6	3 83	p < 0 001
Serum trigly rid (mmol /l)	1 16	0 52	0 04	1 34	0 67	0 05	p < 0 01

NS N statisti al ignificanc

DISCUSSION

In th pre ent tudy th number of pr gnanci tended to be g ate in wo m n with various manif station of IHD than in women in the gen r l population Som of the diff nc w re tati tically signifi ant In dis gre ment with th re ults a previou autop y study fail d t r veal an as ociation betw en number of pr gnanci and deg of oronary ath o l osi [146] How v r th esults of th pr s nt tudy agre with thos of thre oth clini l tu di s [37 108 109] The r ason for a po ibl ociation betwe n numbe of p g nan i and IHD i unknown Influen of a l rg numbe of pr gnan i on v ou body fun tion may be one pos ibl xplanation p y hologic l t u d by a gre t numb of children noth A di p oportionately l rg num be of pontan ous and/o l gal abortion i women with MI has al o to be con id red

Ac ording to sults f om previou studi [84] o l ontra ptive may

be a sociated with an increas d inciden of MI in women In th p nt study no woman was on oral contr ceptives in any of the IHD groups although these drugs were in common us in Göteborg The present series is too small to x clude an association betw en oral contraceptives and MI but in conformity with two p evious reports from Scandinavia[25 60] the r sult of th present series in dicate that oral contra eptives are not a very important factor for development of MI in women

O phor ctomy and spontaneous early menopause have been found to be asso ciat d with an increas d incidenc of IHD[29 1 5 190] An insignif cantly xc ive number of deaths from IHD was also found in a large study of wom n who were radiated becaus of meno rhagia[3] Oliver[130] found that 20 out of 100 women with IHD who w e aged 23 44 wer postmenopausal before the development of IHD Contrary to these results no association betw en oophorectomy or prema ture menopause and an in reas d risk of IHD has been found in some other stu dies [19 134 153] Wink and Hager[207] reported a tudy of 273 women with MI in which 9 women were 50 years of ag o young r These 9 women were all still menstruating when th y had their MI

Th present study supports the view that th re is an association b tween pr mature menopause and IHD A significant number of women reach d the menopau a li r than the women in the general population However all the women with MI ag d 47 or less w re still menstruating when th y had th ir in farct which means that menopause or oophor tomy i definit ly n t an obliga tory factor fo th development of MI at an early ag

As th po t menopau me to be associated with an increas d risk of d v lopm nt of IHD suppl mentation with est ogens might be expected to pre vent IHD The incidence of IHD has been report d to be reduc d in women who w re treated with trogens for postmenopausal complaints[190] but there s ems to be no other tudy whi h ha hown that estrog n suppl mentation reduces th incidence of MI in wom n A controlled prospective study necessary in ord r to valuat this treatment in women

Paradoxi ally th ar more data on th effect of estrogen treatm nt in men Studies on th effect of estrogen tr atment and ts rôle in th pr v ntion of IHD in men have given ontradictory result Favourable[121] unfavourabl[77] a well as in onclus ve results[1] have been reported.

A hypothesis has been advanced that estrogens might influence coagulation a d/or fibrinolytic me han sms in reasing the risk of thrombo-embolic di ase but protect ng from ath ros l rosis and lat r atheros lerotic complica tions[38] Such a hypoth si might explain a possibl incr ased risk of thrombo embol dis a including MI as a result of taking oral contrac ptives a th e trogen compon nt of th ha be n on id red to be th most important in th s respect[2] Th cont ov sial sult of e trogen treatment might al o

be explained in this way Th hypoth is ag ees with the obs rvation by Stam l r et al[170] on est og n tr ted men that the mortality during the fir t period aft r starting strogen treatment wa unexpect dly high while the long t rm urvival increas d. The low in iden e of MI in young and middl aged women ompared to that of men might also be explained in this way

Po sibl mechanisms of st og n on th oagulation system and fibrino ly i hav b en discus ed by Waxl r et al[194] and by A t dt[8] amongst oth rs It has also be n postulated that estrogens int rfere with adrenalin metaboli m or its relea e from th sympathetic nerve[7] Estrog ns a e also known to re duce the s rum chol terol l vel[1 1 190] which i rais d in postmenopausal wo men as shown in th p ent study and al o p viously[77] How ver a protec tive effe t via chol te ol s ms l s plau ibl specially as an inc as d l vel of s rum chol t rol does not m to be associated with an inc a ed risk of IHD in women[77] Nor is it probabl that the ignificantly but mod rat ly increa ed t igly ride level in th po tmenopausal women is a factor of imp rtan e and th low blood p sure in th postmenopausal women hould ontribute to d c ea e rath than in rea the risk of IHD in postmenopausal women

Roman nume l f to th h pt s of thi monograph Arabi num rals f to th f n ll t pp 1 2 128

hospitali ation nor about thos who di d f om MI out ide hospital and only f w compreh n ive data were obtained on ocial facto s in thes women

METHODS

Coll ction of social data. Information concerning marital tatu and place of birth of the women participating in the population tudy wa obtain ed from the R venu Office R gister and by int rview f om the women with MI Information about education number of child n dwelling tandard and socio economic group was obtain d by questionnaire Th subject w re classi fi d into fiv social groups acco ding to Carl son[x x] Group 1 according to thi cla ification i in th p es nt pap pres nt d as ocial g oup I (large scale employ r and offi ials of high or int rmediate rank) groups 2 and 3 a ocial group II (small scale mploy rs officials of lower rank fo emen) and groups 4 and 5 as social group III (skill d and un kill d work r) Subj cts occupi d with dom sti work but no work outsid th home and ubj ct on a pension w re cla ifi d into eparate group Th las ifi ation of th women in th MI group and of th participants in th population tudy was mad by the ame person Information conc rning income during the y ar pre eding the population study o the MI attack was obtained by interview The total in ome was r co ded Pension wa rega d d as in om Status in ongruity wa defin d a either high ducat on of the woman (ontinued aft r l mentary hool) and low incom of h r husband (within the low r 7 de ntil s as found in the popu lation tudy) o a low ducation of th woman (l mentary s hool only) and high income of h r husband (within th upper 3 d centil a found in th popu lation study)

Quantif at on of p ycho ocial str s factors A quantifi at on of psycho social str ss fa tor during th y ar pr ceding the population tudy o th MI attack wa obtain d in an nt rview In a tandardi ed way th subje ts wer a k d about ten group of soc al vent with n gative ignifican e 1) s vere illn of husband 2) n rvou symptoms in husband necessitating onta t w th psychi tri t or r gular drug onsumption 3) husband a chronic alcoholi 4) e probl ms at husband s work 5) legal separation divo ce or husband's d ath 6) s ver illn s or v r behaviour disturban in child 7) last h ld l ft h m 8) sev illness or d th of par nts 9) s ver p o bl m at own wo k 10) mov d to new dwelling A core of ero or on was g ven fo e ry st or g oup Thu an individual ould obtain a maximum tr sor co of ten It v d nt that som st s o s hav had an acut on set during th on y r period while oth rs hav exer sed th ir influ nc during y a Furthe d ta l about the st o scal a e pre ent d els wh r[t]

Ass ssm nt of subjectiv stress exp rience Information about subjective f ling of str s or strain was obtained in another int rview ind pendently of th str or quantification described above and by another physician Th women w re asked wh ther they had had a feeling of stress for a month or long r including tension fear anxi ty or sl ep di turbanc s in conn ction with onflicts in the family at work etc Severe str was defined as a continuo feeling of stress during the year preceding th population study or the MI

Measur ment of personality traits Information concerning per onality t aits was obtain d from tandard qu stionnaires Th pa ticipants in the population study and the women with MI answ red Cesarec Mark s P r sonality Sch dul (CMPS) which i a personality test imilar to Edwards Per sonal Pref r nce Sch dul (EPPS)[46] Both CMPS and EPPS ar bas d upon Murray s theory of pe onality[131]

CMPS consists of 165 qu tions covering 11 pe onality traits achievement affiliation aggres ion autonomy def n of status dominance exhibition guilt fe lings nurturance ord r and succoran After facto analysis th se 1 have be n ombined into five w ighted indi s neurotic s lf ass rtive n rational dominanc aggr ive non onformanc passive d pendency and sociability F exampl neuroti self a ertiven ss was alculat d from th ores of the following primary trait achi vement (x6) guilt f eling (x5) d fence of statu (x5) and agg sion (x4) Two variabl s in the CMPS chieve ment and aggres ion were conside d to measur central a pect of "the o onary behavioural patt rn Type A" described by Fri dman and Ros nman[65] Th behaviou al pattern Type A a behavioural ynd om or a tyl of living haracterised by extrem s of ompetitiven job involvement striving for chi vement aggre iven hast impatience re tl sn and tension

The parti ipant who had had MI wer examined 6 36 months ft the MI attack (m an 18 month) The participant of the population study including tho with AP and coronary ECG were examined at th time of th study

Statisti l m thods

Th hypoth of diff ence in frequenci betw en g oups wa te ted by mean of th binom l di t ibution with no mal approximation when tudying the oci l facto and th ubj tive experien of str In th study of per sonality t ait Mann Whitney s U t t with o re tion for ti was utili ed[166] The st sors w re studi d with a gen rali ation of th t t for trend in contingency tables[72] allowing within g oup matching of ma ri d and singl women Signi ficance of diff ren between g oup means was timat d with Student s t test

Furthe d ta ls of mater l and m thod ar given in Chapte I

RESULTS

Social factors

Social factor in th women with IHD did not differ entially from those in th r f r nc group (Table I) However som tendencies may be not d Thus th re w re comparatively few unmarried women in the various IHD groups Significantly more women had 4 or mor childr n in the MI group and in th group of women with coronary ECG The socio economic status tended to be low r in th women with IHD than in the refer n e group both the socio conomic tatus of th women th mselve and that of th ir husbands (Table II)

Table I Marital status number of children alive plac of birth and education in women hospitalized for MI in wom n with AP and coronary ECG and in a ref renc group of women aged 50 and 54 (%)

	Women with MI n 47	Women with AP n = 29	Women with coronary ECG n 23	Refe en e group n 578
Marital tatus				
Unmarried	2	0	4	8
Marri d	77	79	91	76
Divor d	11	10	4	10
Widow d	11	10	0	6
Numb r of hildr n aliv [a]				
0	20	10	22	21
1 3	61	76	61	72
≥ 4	18**	14	17*	7
Plac of b rth[b]				
County of Götebo g	53	48	48	52
R maining part of Sweden	38	45	48	43
R maining part of Scandinavia	7	3	4	4
R maining part of the world	2	3	0	1
Edu at on				
El mentary school	76	86	87	71
I t rrupted middl hool	7	7	13	8
Middl hool	10	3	0	17
Int rrupt d high hool	2	3	0	1
High hool	5	0	0	2

* $p < 0.05$ ** $p < 0.01$ gn f c ntly diff ent f om the general populat on of wom n

[a]Data on 3 women with MI m g [b]Data on 2 women with MI missing [c]Data on 5 women with MI mi s ng

T bl II Socio conomic group of wom n hospitalized for MI of women with AP and co onary ECG and ocio e onomi g oup of th ir husband a ompa ed to a r f renc g oup of women ag d 50 and 54 (%)

Socio-economic g oup	Women with MI n 44[a]	Women with AP n 28[b]	Women with oronary ECG n 23	Refer n e group n 578
Socio economic group (according to the p of ssion of th wom n)				
I	0	0	0	3
II	20	14	13	30
III	32	34	35	30
Dom stic work only	36	34	35	34
P n ion	11*	17**	17**	3
Socio-e onomi group (a cording to th prof s ion of the hu bands)				
I	5	0	0	14
II	38	48	20	41
III	46	52	75	44
Dom sti work only	0	0	0	0
P nsion	10***	0	5	1

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ significantly diff rent from th gen ral population of women (two tail d t t)

[a]Data on 3 women mi sing [b]Data on on woman mi ing

Th numbe of women working in th hom only wa bout the m in all the g oups whil th number of women re eiving pen ions wa ignifi antly la ger in the g oups of women with IHD than in th f nc g oup Con equently th r w r mo w g rn in the f n e g oup than in the women with IHD Th husbands of th women in the MI g oup w re mo often re iving a pension than th hu bands of th women in the f en group

Th in ome were mila n the variou g oup of women with IHD (Table III) About 70 % in th f ren group nd about 80 % of the MI group w r c iving an in om and/o a pen on. Th dw lling tandard mea ured a numbe of oom was about th am in all th group No significant diff renc in tatu incongruity between hu band and w f defined in the pre ent tudy wa not d (1 % 37 % and 20 % among th ma ried wom n in th groups of women with MI AP and co onary ECG pectiv ly 25 % among th married women in th r f nc group)

Table III Incomes o pension of wom n hospitaliz d fo MI of women with AP and coronary ECG also tho e of a r f r n e g oup of women aged 50 and 54 as w ll those of their husbands if married

	Women with MI	Women with AP	Women with coronary ECG	R ference group
	In ome of the women (Sw crowns)			
Median valu	12000	10000	6000	14000
Income or pension (n)	33/4 [a]	18/28[b]	14/23	388/555
% with income or pen ion	79	64	61	70
	In ome of the husbands (Sw crowns)			
M dian value	30000	28000	25000	30000
Wom n who had a husband with income or p nsion (n)	34/41[d]	21/28[b]	20/23	434/555[c]
% with a husband with income o pension	83	75	87	78

[a]Data on 5 women missing [b]Data on one woman missing [c]Data on 23 women missing [d]Data on 6 women missing

Information was obtained on marital status in 8 women who di d f om MI without being hospitaliz d for suspect d MI Fiv of them we e marri d and 3 unmarried Valid nformation was not obtained about the other ocial factors studied in those who di d outsid hospital or when ho pit lized for r ason other than su pe t d MI

P ycho so ial tressors

As unmarried wom n ar not subjected to all th stres o studied and a th unmarri d women to ome extent are under represented in th hosp taliz d group th number of tressor in marri d and unmarri d women are pres nt ed parat ly As w ll be seen from Table IV the number of tressors tend d to be larg in both married and unmarri d women with MI than in the ref renc group of women Statisti al analysi when married and unmarried wo m n w re match d pa at ly r vealed a ignif cant differ nc between women with MI and women in the gen ral popul tion ($p < 0.05$) Except for stressor nr 7 (last child left home) very stressor wa ov r r pre ented in the MI group The number of wom who reported at l ast one str ssor in th AP group was al o larger than in th fer nc g oup but th was no diff r nce of tatisti cal significance

Tabl IV Women ho pitaliz d for MI women with AP and a r f rence group of women ag d 54 cla sifi d according to number of stre sors (%)

Number of str ssor	Marri d women			Singl women		
	MI n 37[a]	AP n 16	R f rence group n 67	MI n 5[b]	AP n 4	Referenc group n 23
0	43	38	54	60	75	78
1	27	50	30	20	25	22
2	16	12	12	20	0	0
3	11	0	4			
4	0	0	0			
5	3	0	0			

[a]Data on 2 women mis ing [b]Data on 3 women mi sing

Subj ctive stre s expe i nce

A history of psychological stre s according to th woman subj tive f l ing (2 6 in Table V) wa ignifi antly mor often r ported among t the women with MI and AP than in th ref ence group "Seve str ss (5 6 in Table V) wa also ignifi ntly mo e c mmon in the MI g oup (31 %) and in the AP g oup (21 %) when ompared to th ref r n group (7 %) The women with coronary ECG did not diff r ignifi antly from the women in th r f renc group

Tabl V Stres expe ien in women ho pitalized fo MI in women with AP and coronary ECG and in a f rence group of women ag d 50 and 54

Str ss experi n	MI n 42[a]		AP n 28[b]		Co onary ECG n 23		Ref rence group n 578
1 Neve	25		29		52		51
Occa ionally	14		14		13		16
3 Oc a ion lly during th la t five year	14		21		17		13
4 Seve al tim s during th last five y ars	17	***	14	**	13	NS	13
5 Continuou ly during th last year	6	***	7	**	0	NS	3
6 Continuou ly during th last five y a	25		14		4		4

** p < 0 01 *** p < 0 001 ignifi antly diff rent from women in the g n ral population ↑S No stati ti al ignifi an

Data on 5 women mi si g [b]D ta on one woman mi ing

Personality traits

Th aim was hiefly to test the hypothesis that the coronary prone beha viou al pattern Type A as described by Friedman and Rosenman[65 154] was over rep esent d in the women with MI Th two per onality traits studied in the pres nt mat rial which are con id red to measure this behvaioural pattern (agg ssion and achievement) t nded to score higher in the MI group than in the refer nce group The diff ren was significant for aggr s ion ($p < 0\ 05$) but not for a hievem nt Among th other p sonality traits t sted (Table VI) only the factor n urotic s lf assertiveness scor d significantly higher in th MI group than in the refer nce group ($p < 0\ 05$) None of the personality traits scored significantly higher in th AP group than in the ref rence group

Smok rs in the refer nce group tended to core higher for aggression than non smokers (mean scor s 4 4 and 3 2 S D 3 1 and 2 3 respectiv ly $p < 0\ 10$) M an scores for achievement w re similar in smok rs and non smok rs in th reference group (6 8 and 6 3 S D 2 6 and 3 0)

Tabl VI P rsonality traits (scores) in wom n who had been hospitalized for MI and in a r ference group of women ag d 54

Personality trait	Women with MI n 42[a]		Reference group n 68		Signifi canc of diff r nce
	Mean	S D	Mean	S D	
Achievement	7 1	3 0	6 5	2 8	NS
Aggre sion	4 9	2 7	3 7	2 7	$p < 0\ 05$
Dominanc	6 9	3 7	6 4	3 5	NS
Affiliation	10 1	2 5	9 2	9	NS
Defence of status	7 8	3 3	7 3	2 9	NS
Guilt fe lings	8 8	3 0	7 7	3 1	NS
Exhibition	4 6	3 3	4 3	3 0	NS
Autonomy	7 3	1	7 3	2 3	NS
Nurturan	11 6	2 2	12 1	1 9	NS
Order	11 9	2 7	11 9	2 4	NS
Suc oran	8 1	2 6	7 8	2 8	NS
Neuroti self assertiv ness	146 3	34 7	128 6	36 3	$p < 0\ 05$
Rational dominan	6 0	60 3	7 9	55 1	NS
Aggres iv non onforman e	3 4	27 4	7 6	2 4	NS
Passive depend ncy	12 9	30 7	114 8	31 6	NS
Sociability	73 9	16 5	77 1	17 4	NS

NS No stati tical significanc

[a] Data on 5 wome miss ng

High socio conomi tatus has usually been consid red to be associated with IHD in men but th opposite has also be n reported as reviewed by Lehman[109] and Jenkin[86] In a previous study in Göt borg[5] the incomes of m n with MI tended to be low r than those of their cont ols A similar tendency was found in the p ent study of women with MI The incom of th ir hus bands w re about the same in th IHD groups and the ef rence group in the present study The low r in omes of th women with IHD may partly be ex plain d by pre existing heart di ase which may hav fo ced th m to stop working Being pen ioned was mo e common in th women with MI than in th referen g oup Simila ly th ir husbands w mor often r c iving a pen ion

Men born outsid th Göteborg area were signifi antly over r pres nt d among men with MI in Göteborg[5] Thi wa not found for women with MI in the present study

Shekell et al[164] amongst oth di cu sed incongruity of social tatus between husband and wif a a factor a ociat d with IHD With th d finition of status incongruity u d in th p esent tudy no signifi ant diff ren s w re found between women with IHD and women in the gen ral popul tion

MI and AP tend d to be more common in marri d than in unmarried women in Göt bo g which i in agr ment with p eviou obs rvation[197] A high inci denc of IHD in women with many childr n may be an ffect of a larg numbe of pregnan i[11] but st ss experienc be au of many children may al o be an explanation

P ychological tres ha be n consid r d to be a ommon f atur in sub j t with MI both when m asur d as th subj ctiv experien of st s[87] and when measur d as the exposur to str ssor e g d fin d ocial vents o probl ms at home or at work[176] In the pr sent tudy both method of mea su ment w r u d a the reporting of st may have been bia d by th MI attack per s It is rea onabl to uppos that th woman report of defin d tr ss factors i l ss influenc d by th MI attack than h r report of tres ex perienc It i the fo of int re t that both methods gave th ame sults in the p c nt study Exp rience of str a w ll as exposure to ertain de fin d stre sors was mo ommon in women with MI than in th g n ral popu lation of women Wom n with AP who w re participating in the population tudy report d tre s mo oft n than th re t f th population ampl which i of special int t a th two groups w re examin d und th ame con dition

All str r tudied t nd d to be more ommon in th women with MI than in the ref nc g oup ex pt fo th st so number 7 (l st hild l ft hom) Thi di epancy i explained by th fact that th r f n group onsi t d of 54 y a old w m n whil th re was a ang of 40 58 y in the MI g oup

In the present study the last child usually left home when its mother was about 54 years of age. If this stressor had been excluded the differences between the women with MI and the reference group would therefore have been even more prominent. The exposure to other stressors studied increased or decreased continuously or was constant with age.

Factors such as explosive temperament and a high degree of occupational leadership seem to be common in men with MI[62]. The best documented personality trait or behavioural pattern associated with IHD so far seems to be the behavioural pattern Type A. This pattern has been found to be associated with IHD both in retrospective[63] and prospective[157] studies in men and in a retrospective study in women[156]. The use of psychological standard inventories (Minnesota Multiphasic Personality Inventory and Sixteen Personality Factor Test) has failed to show consistent associations between IHD and other personality traits[1,3]. In the present study another standard inventory (CMPS) revealed an increased aggression score in women with MI. It is not known why achievement did not score significantly higher in the MI group as was hypothesized. However, it is well known that women generally aquire lower scores for this trait than men do[38]. It is possible that high achievement is also less common in women with MI than in men with MI.

A somewhat unexpected finding was that neurotic self assertiveness was clearly distinguished between the MI group and the reference group. However, this is not inconsistent with a behavioural pattern Type A in MI prone individuals. It is possible that a tendency to defence of status and guilt feelings indicating a type of ego weakness is just as important as the more overt behaviour Type A in describing the psychology of the coronary prone woman. It can be speculated whether the type of psychic vulnerability revealed by high scores on neurotic self assertiveness is not even more fundamental than the behavioural pattern Type A, the latter thus being viewed as a compensatory reaction in a complicated personality.

The behavioural pattern Type A has been found to be associated with factors such as hypertension, hypercholesterolemia and smoking[65,156]. In a previous study on men in Göteborg smokers scored higher for aggression than non-smokers did[IIII]. Male smokers without known IHD had aggression scores similar to those of men who had MI[4]. In the present series female smokers in the reference group tended to score higher for aggression than female non-smokers did and had scores for aggression similar to those of the women who had suffered MI. The findings of the present and previous studies[65,56] might suggest a central position for behavioural traits in the development of IHD.

Roman numerals refer to the chapters of this monograph. Arabic numerals refer to the reference list pp. 122-128.

Chapt XI

PHYSICAL ACTIVITY IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Call Bengts on

Ab t act Phy i al a tivity both at wo k and during l isur decrea d with ag as found from a population tudy of women Physi al inactivity both at wo k and during l isur had been ignifi antly mo common during the age pe iod 20 38 in women who lat r had an acut myoca di l infarct than in women in th g neral population Th s me wa found wh n comparing lat r period of lif A imil r t nd ncy wa noted in women with angina pe to i though the diff ren mo tly did not ach ignifi ant l vels

Phy ical ina tivity ha be n con id d as on of the risk fa to fo ischa mi h art di s (IHD)[67 164] In th p nt hapt r the physi al a tivi ty in a popul tion sampl of women i ompar d with th physi l activity in wo men with angina pe tori (AP) with ECG chang ugg stiv of IHD (coronary ECG) nd in wom n who had an acute myoca dial infa ction (MI)

MATERIAL

R f e r e n c g o u p The f ren group in th p s nt hapt on i t d of 578 women ag d 50 and 54 who w r participant in a population tudy n Göt borg Sw den[1, 5] and who had about the sam mean ag a th women in the IHD group[I] In ddition a d scription i given of th physi al activity of the participant in all th g g oup tudi d

W o m n w i t h A P Tw nty nin women participating in th population tudy w re found to suffe f om AP as defined ording to Ro[35]

W o m n w th o o n a y E C G Tw nty th women had ECG hang s d fin d a onary FCG[I]

W o m n w i t h M I Forty ven women in Göteb g with an acut MI du ing th y 1968 1970 urvivo on rival in h pital on titut d th MI g oup[I, 9] Women who di d from MI outs d h p t l w n t in lud d no valid information ould be obtain d on rning th p viou phy al a t vity

METHODS

I n t v i w a b o u t p h y s a l t v i t y Th ubj ct w int rview d bout phys l a tivity at wo k and du ing l ure tim Th m phy ici n t rvi w d the total popul t on mpl in luding th w th AP and o onary ECG Th wom n w th MI w int rview d by on of fi phy i an A o d ing to th extent of phy al ti ty th women w re l s fi d into g oup I IV g modifi tion fo women of la fi t on whi h h d p ou ly been u d fo m n in Göt borg[6, 7?] Fo xampl t g oup I fo phy al activity

at work w re assigned women with light office work and no domestic work group II shop work light industrial work or dom stic work including the care of on child group III might include hospital work or dom stic work including th care of two or three childr n and group IV heavy work tog ther with domestic work or *just domestic work including the care of four or more children* A to activity in leisure time examples for group I might be reading or looking at television for group II activity such a walking or cycling for half an hour a day for group III regular activity such as running tennis swimming and for group IV heavy activity or competitive sports

Statistical methods The hypothesis of differ nces in frequenci s betwe n groups was test d by means of the binomial distribution with a normal app oximation

Further details of mat rial and methods are given in Chapter I

RESULTS

Table I cla sifies th women participating in the population study according to age and activity group at work and during leisure It will be seen that physical activity diminishes with age This is more obvious for phy ical activity at work than for physical activity during leisure For th same period of life for in stan e th ir physical activity when they had been between 20 and 38 years of age no differen s wer found betw en th women in the different age groups

Table I Women in diff rent age group classif ed according to physical activity (%)

Activity group	During age 20 38					After age 38					During last year				
	Age group					Age group					Age group				
	38	46	50	54	60	38	46	50	54	60	38	46	50	54	60
Phy cal a tivity at wo k															
I	2	2	1	1	0		2	2	2	1	3	5	3	6	13
II	41	44	38	40	46		60	58	62	73	56	68	69	73	75
III	55	53	60	59	54		37	39	37	26	38	27	28	20	11
IV	2	2	1	0	0		1	1	0	0	3	1	0	1	0
Physic l a tivity during leisu e															
I	10	6	5	3	2		10	12	7	10	17	19	18	13	32
II	78	79	76	83	84		77	73	84	81	72	70	67	80	6[illegible]
III	12	14	19	13	14		13	15	8	9	10	11	15	7	6
IV	0	1	0	1	0		0	0	0	0	1	0	0	0	0

In Tabl II women with variou manif station of IHD ar compared to women in th population sampl ag d 50 and 54 A ignificantly larger number of women in the MI g oup than in th gen ral population we clas ifi d into activity group I for both physicial a tivity at wo k and during l isur during all th lif pe iods tudi d Th was no significant diffe n in physical activity at wo k during the age pe iod b fo 38 betw en women with AP and women in th general population whil women with s dentary work aft r the ag of 38 wer over represent d in the AP group Women who were cl ssified into a tivity g oup I II for activity during l isu during the age period 20 38 were ov r repr ented in the AP g oup ($p < 0.05$) Th physical activity of women with onary ECG had be n imila to that of wom n in the gen ral population b for th ag of 38 A s gnifi antly la g number of women with o onary

T bl II Women with MI (n 40)[a] AP (n 29) and o onary ECG (n 23) and a f ren e g oup of women ag d 50 and 54 (n 578) cla sifi d a co ding to phy ical a tivity (%)

Activity g oup	Phy ical a tivity t wo k				Phy ical a tivity during l isu			
	MI	AP	Co o n ry ECG	R f nc group	MI	AP	Co o nary ECG	R f ren g oup
During ag 20 38								
I	8***	4	0	1	25***	10	4	4
II	69	31	43	39	58	86	87	78
III	22	62	57	59	17	3	9	18
IV	0	3	0	1	0	0	0	0
Aft g 38								
I	17***	7*	0	2	31***	17	32***	10
II	69	59	64	59	64	79	65	77
III	14	34	36	38	6	3	4	13
IV	0	0	0	1	0	0	0	0
During l t yea								
I	22***	14**	26***	4	45***	21	35*	16
II	62	69	48	71	50	76	57	71
III	15	17	26	25	5	3	9	13
IV	0	0	0	0	0	0	0	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ signifi ntly d ff ent fr m th w men in th g n al population

[a] Data on 7 wom n mi ing

ECG were classifi d into a tivity group I for phy i al inactivity during l i ur after th ag of 38 and both at work and du ing lei ur for phy ical a tivity during th year pr vious to th tudy

DISCUSSION

IHD ha been found to be a ommon featur in per ons with sed ntary wo k and in per ons with low physical activity in leisu time[120 127 163 204] Mo t tu di have be n performed on men but phy i al ina tivity s ms to b a sociat ed with an increas d fr quency of IHD in wom n also[120]

Low physical activity both at work and in l isu e tim was more often noted in the women who had MI than in the wom n in the g ne al population in th pr ent study A ignificantly larg r numb r of women with low a tivity during l isure during th ag period 20 38 and at work aft that ag period was not d among th women with AP The phy i al activity during the ag period 20 38 was similar in th women with o ona y ECG and in th r f ren e group of women but low in the wom n with o onary ECG during th y a p eviou to th study

Con lusion must be drawn with caution a int ob rv r variation may hav influen d th r sult It i al o easonabl that wom n with AP du th i a tivity becau of ch t pain Th ignifi antly larg r number of wom n with low physical activity at wo k during th y ar pr viou to th tudy but not during th age period 20 38 y ars in th AP group may indi t this As an atta k of MI i often p ed d by a period of AP[IV] ffort pain may hav r du d th phy ical a tivity during a pe iod befor th MI occu d It i therefo important to not that significant diffe n in phy i al activity betw n wo m n with MI and women with AP on on id and wom n in th g n ral popul tion on th oth w found al ady at an arly ag Thi wa not found f th women with o onary ECG Th ob ervation onc rning the ag period 20 38 favour th vi w that physi al inactivity i a ociat d with IHD in wom n It i tru that th re wa no a ociation betw n low phy i al activity at an a ly g and coronary ECG in women but it is doubtful wh th r o onary ECG a d fined s a manif tation of IHD in wom n[I II]

Roman num al r f r to th hapt of thi monograph Arabi num al f to th ref ren li t pp 122 128

PREVALENCE OF MULTIPLE RISK FACTORS FOR ISCHAEMIC HEART DISEASE IN WOMEN WITH AND WITHOUT KNOWN ISCHAEMIC HEART DISEASE

Calle Bengt son

Ab tra t A larg r number of co exi ting risk factor fo is haemic heart di eas wa found in women who had suff red myocardial infarction than in women in th g neral population. Similar observations w re made when com paring wom n who had angina pe toris o women who had ECG hang s sugg s tive of ischaemic heart diseas with wom n in th gen ral population though th diff ren s w e mall r Diab tes mellitu smoking and high triglyce rid wer p cially common in women who had had myocardial infarction At lea t on of th s risk factor wa p sent in 95 % of th s women com pa d to 48 % of th women with angina pectoris 43 % of women with ECG hang s sugg stiv of i chaemic h rt di ea and 47 % of th wom n in the gen al population It se ms that the p eval nce of a larg numbe of ri k factor even though th fact r may diff only mod ately from those usually found in th population per s acts as an important ri k factor for ischaemic heart dis a e in women.

Conclu ions on rning risk factor for ischa mic heart di ease (IHD) have u ually be n bas d upon tudi of men Th p val n of a la ge num be of o existing risk fa to s factors which have been found to be stati ti cally as oci t d with IHD has be n found to be a common f ature in men pron to IHD[2 59 75] The pr s t chapt r aims to study th co- xi ten e of sev ral ri k fa to in th individual ubj ct in orde to s wh th r an in r as d number of th s is a ociat d with an inc eas d risk of IHD in women also and if the co onary pron woman ha a pe ial con t llation of risk facto s

MATERIAL

R f r n group The r f renc g oup in the p ent chapt r onsi t d of 578 women ag d 50 and 54 who w r parti ipants in a population study in Göt borg Swed n[1,15] and who had approximat ly the same mean ag a the women with IHD[1]

Wom n with angina p to i Twenty nin women participating in th population study w e found to suff r from angina pe toris (AP) as d fined ording t Ro[55]

Wom n with oronary ECG ECG hang d fined a oronary ECG[1] were r co d d in 23 women participating in the populati n study

Women with myoca dial infar tion All pe pl in Göt borg born in 1913 or 1 t who have had an acut ttack of my di l infa ction (MI) hav b en regi t d I th 1 t of Jan ary 1968[1,59] Forty seven wom n who were surviv on arrival i ho pital we regist r d during the year 1968 1970 Anoth 14 women died from a p obabl IHD ttack outsid ho pital

or whil hospitall ed for r asons other than suspected MI[II] 10 of whom were classified as deaths from MI[I]

METHODS

Th methods used hav b en described previously in connection with th analysis of the eparat risk factors [I,V-II] In the present chapter th following definition of risk l vels have b en used

1) blood pressure for the women participating in the population study this means thos on antihyp rtensive treatm nt o those who had a systolic blood pressure value in the seat d position within the upper two decentiles for the women with MI this means those on antihypertensive treatment or thos who had a sy tolic blood pressure value in the seat d position within th upper two decentil s of the forme f male g oup the blood pressure being mea ur d at the 3 month control or at the 12 month control[V]
2) *cholesterol for th women participating in th population study this means* a choleste ol valu within the upper two decentiles for the women with MI this m an a chol ste ol valu within the upper two decentiles of the former femal group aft r adju tm nt for the diff r nce in the serum lipid d t r minations th cholesterol value being m asured ither at the time of the acute atta k at the 3 month control or at th 12 month control[II] for those who died f om an acute attack of MI this means that di tary restri tions and/or lipid reducing drugs had been pre cribed becaus of a high s rum chol ste ol value
3) trigly rid s as for chol st rol for wom n participating in the population tudy and for MI survivo for tho e who di d from an acute attack of MI this means that di tary stri tions and/o lipid r ducing drug had been pres ribed be aus of a high s rum triglyc rid valu
4) smoking ≥ 1 iga tt per day
5) diabet s history of clini al diabet s mellitu or clinically manifest diabet s mellitus at th population study examination or in association with the a ute atta k of MI
6) pregn nc ≥ 4 p gnanci
7) menopau menopaus at th ag of 45 or arli r
8) psychological tr s periods of st ss experienc for a month or longer during th la t 5 y a s which included anxiety nervou ness fear and sl epl s n in conn ct on with onflicts[I]
9) overw ight av rage body w ight [ω] 10 %
10) physical ina t vity t wo k ≤ 5 cale points when summarising s ale points from th e ag pe ods (20 38 aft r 38 nd th y ar p eceding the population study o th MI atta k) according to a scal similar to one previously appli d to men Göt bo g [3 34] but mod fied fo wom n n th p ent study

meaning work mainly in the s at d po ition[11]

ii) physical inactivity during l isure time ≤ 5 cale points when summarizing scal points[301 304] f om thr ag periods (20 38 after 38 and th year p e eding th population study o th MI atta k) meaning mostly r ading and looking at t levi ion[11]

Statistical m thods

Th hypothe is of diff ren in fr quen i of number of risk fa to between g oup wa t ted by means of the binomial distribution with a normal approximation Th hypoth is of diff ren in frequ ncie of the parat ri k fa to s was also t st d in th same way mainly in order to obtain ome in formation about th ir importance as p di tor of IHD

Further d tail of mat rial and method giv n in Chapt I

RESULTS

The p evalen of parat risk facto present d individually fo ach woman who had suff r d MI is hown in Tabl I The number of ri k factor in th ind vidual ubj ct i pre ent d in Tabl II and vi ualized in F g 1

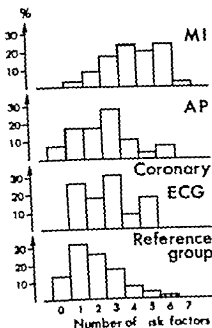


Fig 1 Number of rtain d fin d risk facto in women with MI (n 34 th with ≥ 2 mi ing data are omitt d) in women with AP (n 29) in women with ronary ECG (n 23) and in women in th r f ren group (n 578)

Table I Prevalence of certain defined risk facto s (x) in th individual wo men in th MI groups I high BP II high s rum cholesterol III high se rum triglyc rid s IV smoking V diabetes m llitus VI ≥ 4 p egnancie VII premature menopause VIII = psychological stress IX overweight X = physical inactivity at work XI physical inactivity during l isure time MD mi sing data

Sub j ct no	Age (years)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Hospitali ed becaus of MI survivors on arrival in hospital												
1	37	x	x			x			MD		MD	MD
2	43		x	x	x				x	MD		
3	44		x		x				x		x	x
4	44	MD	MD	MD		x	MD		MD	x	x	x
5	45			x	x	x	x		x			x
6	46	x		x	x						MD	MD
7	47			MD	x				MD			MD
8	47			x	x						x	x
9	48	x	x		x				x	x		
10	48							x	x			
11	48	x	x	x	x					x		
12	48	x		x	x		x			x		
13	49	x		x	x		x					
14	49		x		x							x
15	49			x		x		x		x		
16	51	x		x	x				x	x	x	
17	51	MD	MD	MD						MD	x	x
18[a]	51	MD		x	MD	x	MD	MD	MD	MD	MD	MD
19	52	x	x	x	x		x					x
20	52		x	x	x				MD	MD	MD	MD
21	52								x			
22	53		MD	MD	x		x		MD	x	MD	MD
23	53	x	x	x	x			x	MD		MD	MD
24	53	x		x	x					x		
25	53	x		x	x			x	x			
26	53	x		x	x			x		x	MD	x
27	54			x	x	x		x	x	x		
-8	54	x		x	x		x		x			x
9	54	MD	MD	MD	x		MD		MD	MD	MD	MD
30[a]	54	x	x	x	x	x			MD		MD	MD
31	55	x	x		x							
32	55				x		x	x	MD		MD	MD
33	55	x		x	x		x	x	x			
34	55			x	x			x				
35	55		x	x	x						x	
36	56	x			x							x
37	56	x		x	x		x		x			x
38	56			x	x			x				
39	56	x		x			x		x	x	x	
40	56				x				x			
41	56	x		x	x		x		x	x		x
42	56	x		x	x							x
43[a]	56	x	x	MD					MD		MD	MD
44	57	x			x							
45	57			x	x				x			
46	57	x		x	x				x			x
47	57	x		x	x					x		

[a] Died during the pe od of ho p talization

Table I (contin)

Sub je t no	Ag (year)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
MI deaths outside h spital o whil hospitaliz d fo r a ons other than MI												
1	40		MD	MD	MD			MD	MD	MD	MD	MD
	42	x	x	MD	MD		x	MD	MD	MD	MD	MD
3	48	x		MD				MD	MD	MD	MD	MD
4	51	MD	MD	MD		x			MD	MD	MD	MD
5	51	x	x	MD	x				MD	MD	MD	MD
6	51	MD	MD	MD	MD		MD	MD	MD	MD	MD	MD
7	53	x			MD			MD	MD	MD	MD	MD
8	54	x	x	x	MD	x	x		MD	MD	MD	MD
9	54	x			MD			x	MD	MD	MD	MD
10	56	MD	MD	MD	MD		MD	MD	MD	MD	MD	MD

Women with MI a w ll as women with AP and coronary ECG usually had a la ger numbe of ri k factors than women in the g ne al population Th di tribution of risk facto s wa skewed to th right in th MI group (Fig 1) and to the l ft in th r f r nc group whil th g oups of women with AP and ronary ECG howed an interm diat distribution All women with MI had t l a t 1 risk factor (Tabl II) ompar d to 93 % of th women with AP 100 % of the women with coronary ECG and 86 % in th r f ren group 97 % in th MI group had at l a t 2 i k facto s as compar d to 76 % in th AP group 74 % in th oronary ECG group and 55 % in th f ren group t Th p oportion of w men with a certain minimum numbe of i k facto s wa signifi antly gr t in th MI group than in th f nc g oup Simila tendenci s w r found fo the women with AP and for the women with coronary ECG though all th diff nc s did not rea h ignificant levels It is al o shown in Tabl II that n ne of th wom n in th various g oup had more than 7 f th 11 risk factor studi d

The data of Tabl II and Fig 1 on ern 34 women who had had MI and in whom data conc ning none or at most one variable was missing A s n from Tabl I multipl risk factors w re also ommon in th emaining 13 women for whom a larg r number of data was mis ing and in th wom n who di d from a MI atta k without being hospitalized fo su pected MI

In T ble III th parat i k factors a ranked ccording to the x valu I obtained wh n comparing th p valenc of thes ri k factors in the group of women with IHD with tho in th ref ren g oup This method of ranking is to be regard d only a an att mpt to obtain some information about th importanc of th pa t isk facto Th highest valu in the MI g oup w r ecord d f s rum triglyc rid diabete mellitu and smoki g in the AP group fo previous phy i al in ctivity at wo k many pr gnan i s and psychological st ss nd in the g oup of women with ronary ECG fo high BP

Table II Women with MI AP and coronary ECG and women in th reference group tabulated according to individual number of c rtain d fined risk factors[a] (%)

Number of ·sk factors	Women with MI n 34[a]	Women with AP n 29	Women with coronary ECG n 23	Reference group n 578
0	0	¬	0	14
≥ 1	100**	93	100*	86
≥ 2	97***	76*	74*	55
≥ 3	88***	59***	57**	·9
≥ 4	71***	·1	26*	12
≥ 5	47***	10	17*	5
≥ 6	26***	7**	0	1
≥ 7	3***	0	0	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ significantly different from women in the general population

[a]13 women with ≥ 2 missing data omitted

physical inactivity during l isure and physical inactivity at work Th s values w r cons at ntly lower for the women with AP and coronary ECG than for th women in the MI group

At l t one of three factors diabetes mellitus smoking or s rum trigly ceride valu s whi h w r within the upper quintile of the population sampl was recorded in 38 of 40 women with MI (95 %) for whom data on all three variables w available This is to be compared to 14 of 29 wom n with AP (48 %) 10 of 23 women w th coronary ECG (43 %) and 275 of 575 women in th population s mple (47 %) Thirty s ven of 40 wom n who had had MI (93 %) we e smok s nd/or had ele ated rum triglyc ride values compared to 48 % of th wom w th AP 39 % of th women with coronary ECG and 47 % of the wom in the population sample Both smoking and el vated s rum t iglyc r de lu s w r r orded in 68 % of th women with MI in 10 % of the wom n with AP 9 % of the women with coronary ECG and in 10 % of th wom th populat on s mpl

Table III Ranking of values when comparing th p valences of c rtain risk factor in women with IHD to thos of th women in th referenc group

Factor tudi d	value	Statistical ignifi anc
Women with MI		
High serum triglyc rid	7 70	p < 0 001
Diabet s m llitu	6 40	p < 0 001
Smoking	5 67	p < 0 001
Phy i al inactivity at work	5 64	p < 0 001
High blood p ssure	5 32	p < 0 001
Psychologi al str ss	3 87	p < 0 001
Physical ina tivity during leisure	3 79	p < 0 001
Pr matur m nopau e	2 01	p < 0 05
≥ 4 pr gnanci s	1 60	(p < 0 10)
High s rum hol st rol	1 28	NS
Ov rweight	1 23	NS
Women with AP		
Phy ical ina tivity at wo k	3 94	p < 0 001
≥ 4 pregnanci	2 83	p < 0 01
P ychological st ss	1 99	p < 0 05
High blood pressure	1 62	(p < 0 10)
High s rum triglycerid	1 43	(p < 0 10)
Phy ical inactivity during lei ur	1 33	(p < 0 10)
Pr matu e menopau	1 03	NS
Ov rw ight	0 63	NS
High serum chol t ol	(0 12)[a]	NS
Diabet mellitus	(0 56)[a]	NS
Smoking	(1 12)[a]	NS
Women with coronary ECG		
High blood p sur	3 91	p < 0 001
Phy ical inactivity during leisur	2 61	p < 0 01
Physical inactivity at w rk	2 04	p < 0 05
High s rum triglyc rid	1 70	p < 0 05
Pr matur men pau	1 58	(p < 0 10)
Diabet mellitu	1 44	(p < 0 10)
≥ 4 pregnanci s	1 32	(p < 0 10)
Ov rw ight	0 83	NS
High rum hol t ol	0 52	NS
Psychologi al st	(0 28)[a]	NS
Smoking	(2 43)[a]	NS

[a]Mo omm n in th r f en group NS No stati ti al ignif an

DISCUSSION

With th d finition of risk l v l u d in th p ent hapter ignificant o almo t ignifi ant diff en w found betw en th women with MI and th wom n in th f ren group fo all th vari bl tudied but two rum hol t ol and body w ight In pit of thi high rum hol t rol valu s nd ov rw ight have be n includ d in th p ent analy i a they are usually

taken into consid ration when discussing risk factors

Th risk levels were arbitrarily chosen but wer usually th same in th subjects with IHD as in the reference group Direct comparison i not always possibl For instance the blood pressure is known to fall in conn ction with a MI attack[135 202] and the blood pr ssure m asu ed in association with and after a MI can th refore not be considered as valid for th pre infarction blood pres u of th subject Th highest blood pressure of thos which were r corded either at th 3 month control or at the 12 month control was th refore related to the casual blood pressure of those participating in the population tudy In addition previous antihypert n ive treatment was us d as a omplementary cri terion This complementary criterion is unbia d by th MI attack, but it is poss ible that more subj cts with a high blood pressur w re on tr atment in th MI group as tho e who had a MI may have visit d a docto more often be au of AP or oth r symptom or dis a es which w over represent d in the women with MI[17] For this r a on antihyp rtensive tr atment was not u d as a single riterion for risk l vel of blood pr ssure Nor was the definition exactly th sam fo th women with MI and the women in the population sample when s rum chol sterol and serum triglyc rides w re studied The casual serum lipid valu s w re used for comparison in the population sampl while in the women who had MI the high st of th values m asured either at the tim of th acut MI attack at th 3 month control aft r th attack or at the 12 month control was u d Th decr as in serum lipids known to occur in connection with a MI attack[47 107 202] is thereby consid d to be compensat d

The r sults obtain d in th present study agree with thos of a previous study of m n in Göteborg[184] which showed that the ri k of MI and AP increased with incr a ing number of ri k factor Th combination of high blood pres ure high rum chol sterol and cigar tte smoking ha been consid red to be of particula mportance as p dictors of MI in m n[56 95 184 206] Less is known about th mportance of th separat risk factors for IHD in women Judging from th present study of women high serum triglycerides diabetes mellitus and smoking re part cularly ov r represented in women with MI It is to be not d that at least one of th three risk factors was present in 95 % of the women who had had MI It thus ms that som separat factors are mo over repres nt d than others in women who hav MI but it is evident that the risk of MI n r ases with in a ing numbe of risk factors pr sent

Th sk fa tor patt n of the women with AP seem d to be diff nt from that of th women with MI For xample smoking was much less common in the wom n with AP th n in the wom n with MI The ombination of smoking and high rum triglyc d whi h wa so commonly registered in th women with MI wa no more ommon th wom n with AP than n th population sample However a larg r numbe of risk fa tors tha th population s mple was

found in th women with AP also

The numbe of ri k factors were about the same in the women with coronary ECG as in the women with AP but th "risk factor pattern seemed to be differ nt from that of th AP group and also from that of the MI group A high blood pressure wa a particularly common featur in the women with coronary ECG whi h has brought into consideration the possibility that the coronary ECG as defin d in th present study might be a manifestation of hypertension rather than IHD in women[I]

Th present results s em cl ar in spite of methodological diffi ulties The individual fa tors for th wom n a perh ps only slightly different from thos of the women in th g neral population but ven mod rat differences se m to be important when a number of them are present It s ms that a number of such mod rate differences per se is an important risk factor for th development of MI in women

Roman num al ref to the chapt s of this monograph. Arabi numerals ref to the ef enc list pp. 122 128

taken into con ideration when discussing risk factor

Th risk l vels wer arbitrarily chos n but wer usually the same in the subjects with IHD as in the reference group Dire t ompari on is not always possibl For instan e the blood pressure is known to fall in conn tion with a MI attack[136,202] and the blood pressure measured in association with and after a MI can ther for not be consid red as valid for th pre infarction blood pres sur of the ubject The high st blood pressure of those which were recorded either at the 3 month control or at the 12 month control was therefore relat d to the casual blood pre ure of those participating in the population study In addition previous antihypertensive treatm nt was us d a a complementary cri terion This complementary criterion is unbiased by th MI attack but it is poss ibl that more subject with a high blood pre ure wer on tr atment in th MI group as those who had a MI may have visit d a doctor mo e often be ause of AP or other symptoms or diseases which w re over represent d in th women with MI[27] For thi reason antihypertensive tr atment wa not used as a single riterion for risk l vel of blood pressur Nor was th d finition exactly the same for the women with MI and the wom n in the population sampl when rum chol terol and serum triglycerid s wer tudi d The a ual serum lipid values we e used for omparison in th population sample whil in th women who had MI th high st of the values measured either at th time of the acut MI atta k at th 3 month control aft r th attack or at th 12 month control was us d. Th decrea in serum lipid known to occu in connection w th a MI attack[67,147,202] i th r by con idered to be compensated

The result obtained in the present study agree with those of a previous study of men in Göteborg[84] which showed that the isk of MI and AP incr ased w th increasing number of risk fa tors Th combination of high blood pres su high serum chol st rol and cigar tte sm king has been cons d red to be of particular mportance s pr dictors of MI in m n[54,95,184,206] Less is known about the importanc of the s parate r sk fa tors for IHD in women Judging from th pr sent study of women high s rum triglycerid s diabetes m llitus and moking ar parti ularly over r presented in wom n with MI It is to be noted that at l ast on of th thr e risk factors was p s nt in 95 % of the women who had had MI It thu seems that som separat factors are more ov pres nted than others n women who have MI but it is vid nt that th isk of MI inc as s with inc asing number of ri k facto s pr sent

The r sk facto patte n of th women with AP s med to be diff rent from that of th women with MI For example moking was much less common in th women with AP than in th women with MI The combination of smoking and high s rum trigly r d s whi h was o commonly regist r d in th women with MI wa no more ommon th women with AP than in th population sample However a large numbe of r sk fa tors than in the population sample was

and 180 we e ag d 54 A th ag s of the women in the two age st ata were identi al with those of th m n wh n the men w r examined in 1963 and 1967 respectively comparisons wer made between m n and wom n in thes two age groups

METHODS

The men as well as th women w re examined und r tri tly tandardiz d conditions With a few exc ptions th same t hniqu and m thods were applied. The physical examination of all th men wa perform d by on physician (G T) All the women w e al o examin d by on physi ian (C B) The examination of the men in 1963 and th examination of the wom n w r pe form d in th morning th subject f ting In 1967 th m n w re examin d in the aft rnoon

Blood pr essure det rmination The am te hnique was applied to m n and wom n with th xc ption that th blood pre sure of th m n was ead to th near t 5 mm Hg but in women t th n rest 2 mm Hg M n who w e found to hav sy toli blood pr sur ≥ 175 mm Hg and diastoli blood pre sure ≥ 115 mm Hg in 1963 re ived antihyp rtensive drugs

Serum lipid Th hol t ol level of th men wer d termin d acco d ing to C amér and Isak son[40] Th rum ch l t rol levels of the women w r det rmin d according to a modification of the method d scribed by Levin and Zak[112] Th erum trigly erid l vels of th men w d t rmined acco ding to Carlson[32] and th trigly rid l vels of th women ording to a modifi cation of th m thod des ribed by Loftland[115] Th methods us d in the tudy of wom n gav high val s both for hol te ol and trigly rides than thos us d in th studi s of m n[?] Th xamination of the men in 1967 (when th men w re aged 54) wa not p rf rmed in th fasting tat Serum triglyc rid w th fore not d termin d in 1967

Haemato t Venou blood wa withd awn without sta i with th sub j t in th upin pos tion A mi oha matocrit c ntrifuge was us d Th m an value of two duplicat was u d in th cal ul tion

Smoking hab ts Information onc rning moking habits was obtain d by int rvi w[?] On ga ett wa timated to be equival nt to 1 g of toba o 1 cigarillo 2 g and 1 cigar 5 g tobac o Subj t who smoked ≥ 1 g t ba o/ day w d fined a smok

Alcohol o umpt on Information on rning al ohol consumption wa obtain d f om 54 y a old men and women by int rview Type of consump tion (pirits wine be) and frequen y (Tabl III) w corded

Diab t m ll tus Information wa obtained by i t rview Tho with a history of fasting hype gly mia nd glu uri w d fined a diabeti s In dd tion on woma with pre iou ly unknown diabet but f ting hyp rgly mia and glu suria t th population tudy w on d d to have ma if t di bet

Mental stress Information was obtained by a standardized interview The subjects were asked whether they had had a feeling of stress for a month or longer including tension fear anxiety or disturbances of sleep in connection with conflicts in the family at work etc[2]

Statistical methods Conventional statistical methods were used for calculation of mean values standard deviation (S D) and standard error of the mean (S E) Significance of differences between mean values was estimated with Student s t test The hypothesis of difference in frequencies between groups was tested by means of the binomial distribution with a normal approximation (two tailed test)

Further details of material and method are given in Chapter I and in the descriptions of the population studies of men[102] and women[15]

RESULTS

Arterial blood pressure

Systolic blood pressure was similar in men and women (Table I) There is a tendency towards higher diastolic blood pressure values in men The difference was however slight The distributions of blood pressure were also similar in the two sexes This is shown for the systolic blood pressure in 50 year old men and women in Fig 1

Table I Arterial blood pressure serum lipids and haematocrit in men and women aged 50 and 54

Age	Men				Women			
	n	Mean	S D	S E	n	Mean	S D	S E
Systolic blood pressure (mm Hg) in the seated position								
50	855	138	20 9	0 71	397	138	21 8	1 09
54	799	144	21 2	0 75	180	143	24 1	1 80
Diastolic blood pressure (mm Hg) in the seated position (Phase 4)								
50	855	92	13 2	0 45	397	88	10 6	0 53
54	799	92	12 2	0 43	180	89	12 1	0 90
Serum cholesterol (mg/100 ml)								
50	855	247	4 9	1 47	398	276	41 9	2 10
54	633	270	46 4	1 84	178	284	41 7	3 13
Serum triglycerides (mmol /l)								
50	855	1 26	0 82	0 03	398	1 26	0 58	0 03
54					178	1 39	0 69	0 05
Haematocrit (%)								
50	855	44 9	3 9	0 11	398	39 7	2 95	0 15
54	796	44 1	3 43	0 12	180	40 0	63	0 20

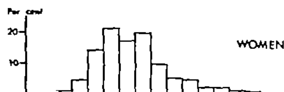
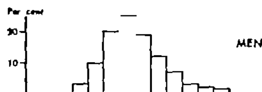


Fig 1 Distribution of systoli blood pr ssure in 50 y ar old men and women The Study of Men Born in 1913 and the Study of Wo men 1968 1969



80- 100- 120- 140- 160- 180- 200- 220-
88 108 128 148 168 188 208 228 mm Hg

In a pr vious ommuni ation[184] it wa shown that hyp rt nsiv women w r mo oft n on antihyperten iv t eatment than hyperten ive men Thus 4 5 % of 50 year old wom n w on antihyp rten ive drugs compared to only 1 6 % of 50 yea old men ($p < 0\ 05$) Excluding thos on antihypertensive drug 3 9 % of th m n and 1 1 % of th wom n had y tolic blood p ssu ≥ 175 mm Hg and dia toli blood p ssu ≥ 115 mm Hg

S um lipids

Mean of s rum h l t ol and rum trigly rid valu fo men and wo m n aged 50 and 54 exc pt fo triglycerid in men ag d 54 whi h w n t studied ar shown in Tabl I Aft r adjustment f the diff ent methods of serum lipid d t rmination in th two studi VI th mean of rum hol te l we found to be 17 mg/100 ml highe in 50 y ar old women and 4 mg/100 ml high in 54 y a old women than in m n of th same g whil th mean of rum trigly rid was found to be 0 28 mmol /l high in 50 y a old men than in 50 y ar old women

H mato it

M ans f ha matocrit valu s in men and wom n g d 50 and 54 r shown n Tabl I Th ha matocrit valu of the men w trikingly high r than th s of th wom n ($p < 0\ 001$)

Smoking h bit

Th we m mok r and more ex smok mong the m n (7 11 II) Th diff ren w tati ti lly ignifi t ($p < 0\ 001$) Smoki g a t g t ba co/day wa lso ignifi antly mor ommon in men than in w en ($p < 0\ 001$) a w ll mong th moking in th gen al population ($p < 0\ 001$ f r g group 50 $p < 0\ 01$ fo g group 54)

Table II Smoking habits in 855 men and 398 women ag d 50 and in 803 men and 180 wom n aged 54 (% M men F women)
Th Study of Men Born in 1913 and the Study of Women 1968 1969

Age (years)	Smokers		Ex smokers		Non smokers[a]		Smoking[b] ≥ 15 g/day	
	M	F	M	F	M	F	M	F
50	56	37	20	6	24	57	20	7
54	54	38	26	6	20	56	17	8

[a]Ex smok rs not included [b]Of the total sample

Alcohol consumption

Men con um d alcohol more often than women did Th difference was statistically significant for spi its and beer (Table III) while wine was consumed as often by wom n as by m n

Tabl III Alcohol consumption in 803 men(M) and 180 wom n(F) aged 54 (%)

Fr quen y	Spirits		Wine		B r	
	M	F	M	F	M	F
Every day	3 ***	1	1 NS	2	36 **	14
S veral times a w ek	14	2	5	4	22	14
Onc a week	28	–	13	12	15	22
Once a month	22	18	19	36	6	16
S ldom or n v r	32	73	61	47	0	34

*** $p < 0.001$ s gn fi antly different from wom n NS No stati tical s g lfican e

D abet s m llitu

Th pr val n of d abet s mell tus was sim lar in 50 y ar old men and women (0 8 % in th m a d 1 3 % in th women) and in 54 year old men and wom n (1 0 % and 0 6 % r spectiv ly)

M tal str s

Wh n int rv w d in th same tandardi d way 54 y ar old m n reported cont nuous xperie of m tal stre s during th y a pr vious to th tudy more oft n tha 54 y ar old wom n (18 % as compared to 7 % $p < 0.001$)

DISCUSSION

According to results obtain d in previou studi s[47 50 52] blood pressure has been foumd to b high r in men up to the ag of about 50 and th n high r in women but with no marked differen s at any ag Blood pr ssur in men and women aged 50 and 54 may thu be xpected to be about th ame and thi wa also found in the Göt borg studie Th light diff n not d for dia toli blood p sure may be du to int observer variation Statistical analysi has not been performed for blood pre su as the conditions w r not xactly th same However th results ar l ar enough to show that ther ar no important diff rence in blood pressur b tw n m n and women at the e ag s which can expl in a higher incid n of MI in men It i po sibl that th lightly high r blood pre sur in men than in women of younger ag may ontribut to th s x differenc in MI but the diff en is probably too mall to be of importanc

Women w re mo oft n on antihyp rten ive tr atment than men w Poss ibly part of this diff r n may b explain d by th tim laps between th tudi s of men and women How v r if th re i a al diff renc this an probably not explain much of th sex differen e in MI specially a m an and distribution of blood p e sure w re imila in pit of the diff en in number on antihype ten ive t tm nt

A diff rent m thods fo d t rmination of hol t rol and trigly rid l v ls had been u d for men d women tati tical analy is wa not p rf rmed and con lusions from ompa isons between th two exe in thi sp ct must be drawn with caution Aft r adju tm nt fo diff r nt m thod of s rum lipid det rmination serum chol t r l was found to be about 5 15 mg/100 ml highe in wom n than in men in th ag s tudi d while s rum triglyc rida were about 0 30 mmol /l high in th men Th s ex diff enc agre with tho not d in Stockh lm Sw d n[34] Thus there ar probably no diff ren e in rum lipid at th ag whi h an xplain th ex diff r in MI S rum chol t rol lev ls have be n found to b high l men than in wom up to the g of about 50 and th n higher in women whil rum trigly rid l l em to be highe in men both bef and aft r th g f 50[34 53] Th high ch l st rol and trigly rid valu s in men bef th ag of 50 may contribut to th diff ren in in denc of MI betwe n th x s but a for blood pr ur th ex diff r n s in young r g probably too light t be of g at importan e

The ha matoc it valu s w m h high l men than i women A high ha moglobin valu ha b dis us d as a i k f to fo IHD in men[7 ??] A multivariat analy i of risk f tor fo IHD in me in Göt bo g[??] did t show a ignifi t l t on hip betw n ha matocrit and MI Ha mato rit wa not a ried out gul ly in th pr ent of wome who suff r d MI H emoglobin valu w r not high in wom n who uff red MI than in wo n n

in the g n ral population Although there em to be no d finit association between a high haematocrit value and MI in ith r s x the diff rence in ha matocrit between th sexes might explain som of the sex diff rence in the in id nce of MI

In agreement with pr vious tudies[22 48 96 179] smoking wa much mor com mon in men than in women and the mal smoke s were mor often h avy smok rs The sex diff r nc in smoking habit may be one r ason for the sex differ nce in MI

Al ohol ha be n discus ed as anoth r pos ible risk factor for MI[185 192 206] In th pres nt study men onsumed alcohol mo oft n than women did High r alcohol consumption in m n may be one reason for a higher incid n of MI in m n

A similar pr valence of diabetes mellitu in m n and women wa found in Göt borg Thi is in agr ement with previou stud s[65] which indicate that the prevalenc of diabetes is about th sam in the two sex up to the ag of 50 The ex diff rence in the in idence of MI an thus not be xplained by sex diff rence in the pr val nce of manifest diabete

Som previous r ports hav pointed out that diab tes is more oft n a ociat ed with MI in wom n than in m n[38 61 104] This ag with the observation in Göteborg Thu 15 % of women ho pitalized for an acut MI had manifest d a bet [VIII] compared to 6 % of m n with MI[201] The diff r nce wa not tati tically signif cant Based upon figures for th preval nc of diabet in men and wo m n with and without a previou MI and on figu es for the inc den of MI in th population[III] th in d nce of MI in non diabetic m n in Göt borg ould be timat d to be about 10 times that in non diabeti wom n while the incidence in mal diabetic was found to be about 3 times that in f mal diabetics The inc dence in d abeti women wa found to be about twice that in non diabet men

It is difficult to valuat th finding that p ychological tress was a mor ommon feature in men than n women as the way of lif i so diff nt in th two ex The diff ren in stress experience report d thu need not n ce ar ly mean that m n a mor expo ed to p ychologi al tr s than women Howev r if th r s a r al x diff r nce this may xplain som of the ex d ff ren th in den of MI

B havioural t its w studi d by mean of a qu stionnair (CMPS) in th p e nt t Aggr ss on wa found to be significantly mor ommon in women who had uff d MI tha in th g ne al population of women Th ggr ssion o wa h gh r n a randomized sampl of 52 y ar old men in Göt borg than in th ampl of 54 y ar old wom n (Table IV) S or s for achiev ment nd domi n al o med to be high in m n (Tabl IV) whil other tra t tud d g v milar co n men and women Smok rs

and non smoker w re studied s parately as smok r wer expect d to s s higher[4] Aggr ssion and achievem nt are con id r d to measure th corona, pron behavioural pattern Typ A [63] as wa previou ly di cussed in Chapt r X It is possibl that there are ex diff r nc s in b havioural traits which might xplain om part of the sex diff r nc in th incidenc of MI

Tabl IV Me n s o s (CMPS) fo agg ssion achiev ment and dominan in mal (M) and f mal (F)[2] smok r and non smok

	Smok rs M	Smok rs F	Non smokers M	Non smokers F
	n 8	n 30	n 42	n 38
Agg s ion	5 2	4 4	3 8	3 2
Achievement	7 4	6 8	7 1	6 3
Dominanc	8 5	6 1	7 5	6 7

Tak n tog th som diff renc in isk facto w found betwe n men and wom n The w diff ren in smoking habit alcohol onsump tion ha matocrit and p obably in str s experienc Th e might be diff renc in personality t ait No impo tant diffe nc we e found wh n om paring a numbe of th ri k facto It m unlik ly that ex diff ren es in known risk f ct r explain more than some part of th diff ren e in th in d n e of MI b tw n m n and women Thu th r i p obably om unknown sex linked f ctor o fa to p ote ting women from MI in young and middl ag

Roman num ls f to th hapt of thi monogr ph Arabic nume al ref to the ref rence li t pp 122 128

in the g neral population Although there s me to be no definit association betw n a high ha matocrit valu and MI in eith r ex th diff rence in haematocrit between th sexes might explain some of the x diff rence in th in id nce of MI

In agreement with pr vious studies[22,48,57,127] smoking was much mor com mon in men than in wom n and th mal smoke were mor often h avy smokers Th sex diff ren in moking habits may b on reason for the sex diff r nce in MI

Alcohol has been discussed as another po ible risk factor for MI[185,192,204] In th pr s nt tudy men consum d al ohol mor oft n than women did Higher alcohol con umption in m n may be on reason for a high r incid nce of MI in men

A simular preval nce of diabetes mellitu in m n and women was found in Göteborg This i in agr m nt with previou tudi s[69] which indicat that th preval nce of diabetes is about the same in the two sexes up to the ag of 50 Th x difference in th incid n e of MI can thus not be explain d by sex diff r nc in the pr valenc of manifest diabet s

Som pr vious r ports have pointed out that diabet s i mor often associat d with MI in women than in men[88,68,104] This agr es with the observation in Göteborg Thus 15 % of wom n hospitalis d for an acut MI had manifest d a bet[VIII] ompared to 6 % of m n with MI[201] The diff ren was not tatisti ally gnificant Ba d upon figur s for th preval nce of diabetes in men and wo men with and w thout a pr viou MI and on figure for th incidenc of MI in the population[III] th in id n of MI in non diabetic men in Göteborg could be stimat d to be about 10 t me that in non diabetic women whil the inc d nc in male diab ti s was found to be about 3 tim s that in femal diabeti s The d nc in diabeti women wa found to be about twi that in non diabetic m n

It difficult to evaluate th finding that p ycholog al str s wa a mor common f ature in m n than n women as th way of lif i o differ nt in th two sexes Th differ n e in str exp rien report d thus n ed not nec ar ly m an that men ar more exposed to psych logi al stress than wom n Howev r if th e i a r al x d ff r n thi may explain om of th s x d ff en n th nc d n of MI

B havioural t a t w studi d by me ns of a quest onnaire (CMPS) in the p s nt r[I] Aggr on was found to b significantly mor common in women who had uff d MI tha in th g n ral population of women Th aggre s on s o was h gh r in a randomi d sampl of 52 year old men in Göt borg than in th ample of 54 y a old women (Table IV) Score for achi v m nt a d dominan l o m d to be high in men (Table IV) while other tr t t di d gav mular co s n men and women Smok rs

ag 60 with primarily un omplicated AP di d within 8 y a s[36] Th e se m to be ome additional factor which c us s MI in men with AP but ldom in women with AP

Smoking might b such an additional fa to Smoking is more ommon in men than in women and mor ommon in subj t with MI than in subj t f f om MI irrespe tiv of ex[VII] Th s ms to be no di tinct ove rep n tation of smok r in ubj ct with AP of eith r s x Con equently it m that smoking i of no o little importance in th d velopment of AP but i of gr at importanc in the dev lopment of MI This may be another way to explain why th pr valen of AP i about th am in both ex whil MI i much mo common in m n than in wom n

A number of fa tors w r found to be mo ommon in women with IHD than in women in th gen al population Most of th fa to s tudi d a summarized in Tabl I

Tabl I Comparison betw en women with IHD in the pr nt eri and women in th g n ral population on rning s me fa tors studi d

Facto tudi d	Wom n with MI	Women with AP	Wom n with oronary ECG
P eviou MI o AP	+		+
Intermitt nt claudi ation	+	+	
Gall ton or nal ton di ea	+	+	+
Art rial hyp rtension	+	(+)	+
Hyp r h le t ol mia			
Trigly rid mia	+	(+)	+
Smoking	+		
Diabet s mellitu	+		
Glu o intole an [a]			?
Dec as d insulin respon [a]	(+)		?
Many p gnan i s	(+)	+	+
Pr mature men paus	+	+	+
Social fa to			
M ntal stre s	+	+	
Certain pe son lity t ait	+		?
Phy ical ina tivity	+	+	(+)
Overw ight			
High ha mogl bin valu			
La g numb of ri k fa tor	+	+	+

\+ tati ti ally signifi ant diff n (+) tati tically insignificant tende cy — no tend ncy noted ? omparative naly i not carri d out

[a]In non diabeti women

Body weight and haemoglobin are included in Table I although they have not been presented separately in previous chapter. Women with AP were slightly shorter than women in the general population; apart from this there were no significant differences in body weight or body height. Being overweight was thus not found to be a risk factor for IHD in women in the present study, which agrees with the results from Framingham[100]. A raised haemoglobin concentration has been found to be associated with IHD in men[2, 99] but not in women[99]. The haemoglobin concentrations of women with IHD in the present study were no higher than in women in the general population.

No association was found between high serum cholesterol values and IHD in the present study, while there was an association between high serum triglycerides and IHD[77]. There seems to be an association particularly between cholesterol and IHD in men[71, 106] and between triglycerides and IHD in women. Such a difference between the sexes may partly be explained by hormonal effects on the serum lipids in women. Estrogens decrease the cholesterol level while they may raise the triglyceride level as is noted for instance during pregnancy or estrogen administration[7, 66].

Women who suffered MI were not asked about previous alcohol consumption. High triglyceride values have been reported in alcohol consumers[99] but the high triglyceride values of the women with IHD in the present study can probably not be explained in this way.

The number of pregnancies and childbirths tended to be greater in women with IHD than in women in the general population[77]. This may be explained in many ways. Pregnancy may predispose to IHD, for instance by increasing the serum triglycerides. As the experience of stress is also associated with IHD and as a large number of children may be a stress factor, the stress experience rather than the number of pregnancies might be the risk factor. Unmarried women tended to be underrepresented among the women hospitalized for MI and in the women with AP. This may be explained by a smaller number of pregnancies among these women. Or is a man to be considered a risk factor for IHD in a woman?

If the influence of estrogen on serum lipids is confusing, the association between estrogens and IHD is even more confusing. It seems that treatment with oral contraceptives or estrogen administration for other reasons may induce IHD. On the other hand long term administration of estrogen, the sex difference in incidence of MI and the high incidence of MI in women with premature menopause indicate that estrogen also protect from IHD. The possible influence of estrogens on IHD was discussed in Chapter IX. It was considered probable that estrogen may be of importance for the development of thromboembolic disease but give long term protection from IHD, maybe by protecting from the atherosclerosis of the coronary arteries. Atherosclerosis of

the oronary art ri s has in an autopsy study be found to be mo e promi nt in men than in wom n[174]

It ha b en much d bat d wh the smoking is a cau tive factor fo MI or if smoking is linked to som oth r factor which is th causative on [III] Among personality traits tudi d aggre ion tend d to be mor common in smokers than in non mok rs and mo e common in subje ts with MI than in subj cts who had not suff d MI[I] On the oth ha d no a sociation was found betwe n triving for man pation and smoking For exampl f mal mok rs wo ked outsid th hom no mor oft n than th f mal non smoke s thei in om s w imilar and they eport d pr ious mental stres with similar f equency

Many other xample of possibl co variation of variou risk fa to s could be st t d Howev r th present report did not aim to di cuss such co variation in detail As ha been mphasiz d[I] risk fa tors me n statisti al lation hips and say nothing onc rning causation Mor definit information o c rning ausation an only be obtained f om p v ntive studies with int r vent on on ri k fa to [n?]

Th e was also a diff renc in numbe of risk f to between th women with MI and th wom with AP o coronary ECG which may probably partly be explain d by varying accuracy con rning the crit ria of thes thre mani f stations of IHD Probably th number of fal positives is larger among th women with AP or coronary ECG than among the women with MI An admix tur of fals po it v in the groups of women with AP or coronary ECG may have dimini hed the diff rences between the wom n with AP or " oronary ECG and th women n th g n ral population It also ems that women with MI are d ff rent from tho with AP Perhap some factors c u oronary athe o cl ro i om dditional factor AP and still oth MI

Th re mu t be ome reason o r ons fo th highe prevalenc and in i d n of MI in men than n women Smoking wa di cuss d a on fa tor whi h m y explain some of th ex diff rence in MI H weve it cannot explain mor than p rt of th diff n as hown in Tabl II A rough timat of th in i d c of MI in moking nd non moking men and wom n ba ed upon inciden data[III,40] data on moking from th population tudi of men and women aged

T bl II Approximat nnu l inciden of MI pe 1000 smoking and non moking men nd women g d 50 54 i Göt borg

Category	App oximat annual inciden e f MI
M l mok rs	4 5
M l non mok	1 0
F mal smok	0 9
F mal non mok rs	0 1

50 and 54[I II] a pr vious report on smoking in men with MI[49] and data con cerning moking in the pr sent study of women with MI[III] is presented in this table MI was more common in male smok rs than in female smokers The in cidenc was approximat ly similar in male non smoke s and f mal smokers

Experience of mental stres was mor often reported in men than in women but this difference can probably only explain a small part of th sex difference in the incidence of MI Men consum d alcohol mo often than women did Haematocrit was high r in men S rum triglyceride l vels s emed to be higher in men than in wom n both before and aft r the age of 50[III 34 93] High erum triglyceride l vels wer common in women who had suff red MI[II III] A sex diff ren e in serum triglyc rides might th r for be of importance The age trend with higher blood pressur and higher serum cholest rol l vels in men than in women up to th age of about 50[III 34 93] may pos ibly also ex plain som of th sex diffe ence in the in idence of MI Otherwis th re were no important sex differ nc in risk factors

Summarising differences in various risk factors it s ems probable that they may explain part of th sex diff rence in MI How v r the mo t promi nent difference between th exe in this respe t is obviou ly still the simple fact that men ar men and wom n ar women

Roman num ral r f r to the hapter of this monograph Arabic numerals r f r to the ref r n e l st pp 122 128

GENERAL SUMMARY

The present study wa bas d upon a ample of the femal population and on a s ri s of women in th s me population who suff r d myocardial infarction (MI) during a defined period of time Women in th popul tion sampl with symptoms of angina pe toris (AP) and ECG chang s ugg stive of i cha mic heart dis ase ('co onary ECG) were studied separately Comparisons were made between women with variou manife tations of ischa mic heart di ea (IHD) and women in th population sampl who were fre from th IHD mani festation studied. A comparison was also mad betw n th mal and female populations

MI was mor ommon in men while th prevalen e of AP wa imilar in m n and women A history of pr vious MI and/or AP wa ommon in women who suffer d MI Arterial hyperten ion high erum trigly ride smoking diabete mellitus mental str s and physical ina tivity wer ommon features in thi g oup of women Prematur menopaus was mor common than in wo m n in the g n al population However non of th women who had a MI be fore the ag of 48 had r ached th m nopaus at th tim of th MI Co exist nc of several risk factor chara teriz d th women with MI

Th char ct ri ti s of th women who had AP we imilar to tho e of th women wh had MI with the ex ption of smoking whi h, in ntrast to women with MI wa no more ommon in women with AP than in thos fr from AP in the population

Non of th women in th population sampl had ECG chang ugg sting a previous MI Th ECG hanges d fined as oronary ECG almost ex lu iv ly ompri ed ST dep sions and T inv rsion Hyperten ion was a common f tur in th women with co onary ECG whi h might sugg t that coronary ECG a defined in th pr ent study is a manif station of hypert nsion ther than IHD in wom n

Smoking and al ohol onsumption were mo common in men than in women Ha matocrit was high r in men Men mo oft n eported xperienc of mental str s How ver diff n in e tablish d isk fa tor annot explain mo than part of th ex diff renc in th incidence of MI Th r seems to be some oth ex linked facto or fa tor p ot cting women from MI in young and middl ag

ACKNOWLEDGEMENTS

I would like to express my gratitude to Professor Leif Hallberg, who initiated the present population study of women and provided excellent conditions for its realization. His advice and constructive criticism concerning the analysis and presentation of the data has been very valuable.

I would also like to thank Associate Professor Gösta Tibblin, who in addition to proposing the inclusion of women with myocardial infarction in the study and giving valuable advice and criticism, has together with Professor Lars Werkö and Associate Professor Lars Wilhelmsen provided excellent conditions for the study of women with myocardial infarction. Thanks are also due to Lars Werkö and Lars Wilhelmsen for their interest and valuable comments on the manuscript. Valuable comments on the manuscript were also received from Professor Sven Lindstedt, Associate Professors Per Björntorp, Gillis Johnsson, Ulf Smith, and from Margaret Macpherson (London) who together with Mr John Gulliver revised the English text.

Carrying out the population study of women as well as the study of the subjects who suffered myocardial infarction entailed extensive team work and I am very grateful to my co-workers in the population study and the personnel at the Post MI Clinic: Associate Professor Göran Rybo, Dr Göran Blohmé, Dr Tore Hällström, Dr Elisabeth Tibblin and Dr Hans West, being carried out various important parts of the population study. Special thanks to Professor Sven Lindstedt and Dr Elisabeth Tibblin for handling the laboratory analyses. Mrs Gullbritt Palm carried out the statistical analyses. Mrs Mona Mattiasson contacted all the participants in the population study and her enthusiasm made possible the high participation rate. Assistance received from Mrs Kerstin Claesson, Mrs Alice Hast, Mrs Viola Karlsson, Miss Lilly Olsson and Mrs Margit Pettersson has been highly appreciated.

The women with myocardial infarction in the present study were examined by Associate Professors Gunnar Angervall, Gösta Tibblin, Lars Wilhelmsen, Dr Dag Elmfeldt and by myself with skilful assistance from Miss Britt Holmberg and Miss Thelma Wikand amongst others.

The library staff have been very helpful. I would especially like to thank Mr Rutger I g n, who by means of MEDLARS helped me to cover the literature in this broad field.

The figures of this monograph were prepared by Mrs Viola Karlsson and by Mrs Ulla Attmås, who also prepared them for offset printing.

Valuable secretarial assistance has been obtained from Miss Lena Arvidsson and Miss Anna Lena Johansson amongst others, as well as from Miss Gunnel Henricsson, who also typed the final version for offset printing.

The population study was supported by grants from R and V Andréen's Foundation, AB Astra, the Mutual Group Life Insurance Company Förenade

Liv th Foundation of th Jubil e Clinic Göteborg the Swedish Nutrition Foundation the Swedish Margarine Manufacturers Association and th Swedish National Associ tion against H art and Che t Dis as s Premises were made available by the City of Göteborg

The Post MI Clinic was supported by grants from th Mutual Group Life In uranc Company *Förenade Liv* and the Sw dish National Association against Heart and Chest Dis ases

My thanks also to AB Hässl who via its R arch Director Dr Ivan Öst holm and its Medical Dir ctor Associate Professor Gillis Johnsson supported my work.

La t but not lea t sincere thank to my wif who b sid supporting me in my work assist d in almo t very part of it

REFERENCES

1 ACHESON R.M Th ti logy f ronary heart di ease re i from the pidemi logical tudy i Yal J Bi l Med 35:143-170 1962

2 AITKEN J.M LORIMER A R F LAY HART D LAWRIE, T D V & Smi h D A The ff te f oophore tomy and long term oe ranol therapy on the serum lipid of middl -aged wome Cli Sci 1 597-603 1971

3 ALDERLOV M R & JACKSON, S.M Long term f llow-up f patien ith menorrhagia treated by i radi tion B i J Radi l 4 295-298 1971

ANDER, S Prognosf ktore f t l ta röka i grupp man med hjärtinfarkt Rapp rt från Psyk logi ka i i ion Got borgs Universi t No 24 Go borg 1973

5 ANDER, S ANDERSSON L, LINDSTROM B HANSE, K & TIBBLIN G P yk sociala f kt re id hjärtinfarkt Läka tidn. 68 3695-3699 1971

6 ARMSTRONG A, DUNCAN B OLIVER M.F JULIAN D G DONALD K W FULTON M LUTZ W & MORRISON S L, Natural hi tory f ac te co onary heart ttacks A community tudy Brit Heart J 34 67-80 972

7 Arterial hype t i and ischaemi heart di eas Preve tive asp ts Wld Hlth Org.techn Rep Se No 231 1962

8 ÅSTEDT B Low fibrinolyti activity of veins during treatment with thinylos tradi l Acta b tet gynec scad 50 279-283, 1971

9 ÅSTRAND I ARESKOG N H BLOMQVIST G BJERKELUND C CARLSTEN A, FURBERG C OPENTH K, K HANSEN F KALIJER L, KALLIO V MALMSTROM G NORDGREN L, PUNSAR, S PYORALA K, & TRULESTED O The "Minnesota Cod f ECG lassifica ion Adaptation to CR lead and odification f the od f ECGs rec rded during and afte ercci Acta med scand Suppl 481 1967

10 ASTRUP P Some physi logical and pathological ffect of od rate carbon mono id xposur Brit ed J 447-452 1972

11 AUERBACH, O HAMMOND E.C & GARFINKEL, L: Sm ing in relation t therosol ro i f the coronary art ri Ne Engl J Med 273 775-779 1965

12 BAGDADE, J.D BIERMAN, E L, & PORTE Jr D The ignificance f basal insulin l vel i the valuation f the insuli spons to glucos i diabeti and nondiabeti subj ts J lin Inve t 46 1549-1557 967

13 BAILEY R,R & BEAVEN D W A retr pective analysi f 500 pati ts with acute myocardial infarction N Zeald med J 67 479-486 1968

4 BASSETT D R, & SCHLOFFREN O Bl od lipid and lipoproteins gluc tol rance and plasma insulin resp se in Chine me with and with ronary heart disease i Hawaii Israel J med Sci 5 666-670 969

15 BENGTSSON C BLOHMÉ G HALLBERG L, HALLSTROM, T ISAKSSON B KORSAN-BENGTSEN K RYBO G TIBBLIN E TIBBLIN G & WESTERBERG H. The S udy f Wom in Gothe bu g 1968-1969 pop lation udy General d ign purpose and sampling lts Acta med scand 193 311 318 1973

6 BERCHTOLD P BJORNTORP P GUSTAFSON A, LINDHOLM B TIBBLIN, G & WILHELMSEN L, Glucose tolerance plasma ins lin and lipid i relation to adipos i cose cell lari y i en afte yocardial infarc i Acta med cand 191 35-4 1972

17 BI-ÖCK, G Som comme ts on th epid i logy f ronary disease Bibl Kat t Di ta 6 24 1964

18 BLACKBURN H KEYS A SIMONSON E, RAUTAHARJU P & PUNSAR, S The lectrocardiogram i popula ion ud A la f on ion system Ci ula 21 1160-1175 960

19 BLACKMAN N S & KOLODNY T Acu myocardial infarc ion in wome und fif y years f age N Y St J Med 6 3079-3082 96

20 BLOHMÉ G In ra eous gluco l rance in 50- ear ld women. A pop lation udy In preparation

2 BLOHMÉ G & WALDENSTROM, J Earl sulin respons to i ra eous gluc se inj tion i 50-year ld wome popula on ud In prepara ion

22 BOALT G En soci log på svo ha f l Sterns und om te d ani Ins Om bab i S rige J l ang rif 1915- 965 pp 173- 96 Svenska T taks Ak i bolaget Stockhol 965

23 BONFIGLIO T A BOTTI R E, & HAGSTROM, J W C Coronary arteriti occlusion,and myocardial inf ion due lupus rythema sus Am Heart J 83: 53-158 1972

2 BOTTIGER L. E LAPLJON L.A. Stockholm Pro pective S udy II ai års uppföljning Läkartidn 69 640-4644 972

25 BOTTIGER L.E & WESTERHOLM B Oral co racep ve and thromboemb li dieeas Acta med scand 90 55- 63, 197

26 BRATT L. OLÉN Å CRONVÍ B TRYDING N A diet d te ion campaign in S thern Swede R ults f 69 000 sam na ons Acta med cand 76 553-561 1964

27 B EKENRIDG A WELDON T COLLINS T FRASER R Gl co l rase i hype tensive pa i l g ra d ar th rap ion 6 -6 967

28 BRI KER O.N Bl af rt d l ai card la d ho melli us rch.In Card l 4 30 380-396 968

29 BROEDERS G.L.B De b kenl van de endocriene functi van be varicose e d preventi van coronaria-sclerose d and lipo gevolgen van voortijdige en tratt Ned T Geneesk 113:13-16 1969

30 BROWN D.F AYERS V.R. & BOYLE, J T Glucose-lipid interrel tionship i ischemi heart disease Circulation 28 696-697 1963

31 BROWNLEE, K.A. Statistical theory and methodology in sci nce and ngine ring, 2nd ed John Wiley & Sons Inc New Y rk London and Sydney 1965

32 CARLSON L.A. D t rmination f serum glyc ride Acta Soc Med upsalien 64 208-213 1959

33. CARLSON L.A. & BOTTIGER L.E. Ischemic heart-disease in relation to fasting value of plasma tr glyceride and chol t rol. Stockholm Prospecti Study Lancet 1 865-868 1972

34 CARLSON L.A. & LINDSTEDT S The Stockholm Prospective Study I. The initial value for plasma lipids Acta med.scand Suppl 493 1968

35 CARLSSON, G Socialgruppering (Stencil) Lund 1956

36. CARLSSON G Social mobili y and la tru ure CWK Gleerup Lund 1958

37 CERASI, E. & LUFT R. The plasma ins lin response gluc se inf sion in healthy bjects and i diabetes mellitus Acta endocri (Kbh) 55 278-304 1967

38 CEDERLÖF Z. HAREL, S Rö ning psykogena be med frågeformulär rot kaik Manual till CEPS Stockholm Testforlaget, Stockh lm 1968

39 CHAIT A. MANCINI, M. FEBRUARY A.S & LEWIS B Clinical and metaboli tudy of alcoholi hype lipidaemia. Lancet 2 62-64 1972

40 CRAFÉN, K. & IHALSHOF B An evalua on of the Theorell me hod f the d termination f tal se m ch l sterol Scand.J clin.Lab Invest. 1 213-216 1959

1 DALGAARD J.B & GRIGORIEK, N Koronartromb se unde ersøl se ikketrop ien Nord.Med 81 273-279 1969

2 DAWBER T R, MOORE, F E & MANN G V C ronary heart diseas in h Framingham Study Amer J p bl Hlth 7 Suppl 4-24 957

3 DOMINGO, L. ESPINOSA, C ALONSO R.G ESTADELLA S Frecuencia de arterosclerosi coronaria y aórtica en la ciudad de Mérida Influencia d l sexo la ed dad la diabetes y la hipe tensión arterial. Re Inve lin. 6 397-41 964

44 DOYLE, J T DAWBER, T.R. KANNEL, W.B KINCH, S.H. & KAHN H.A. The relationship of igare te sm king to coronary heart disease The second report of the combined experience of the Albany NY and Framingham Mass tudi J.ame med.Ass 190 886-890 1964

45 EHRENDAL, J DAHL, E.G. & NILSSON J.A. Plasma lipids and lipop teins i pat en wi h ocardial infarction and in oon l m orial. Acta med scand 187 353-363, 1970

6 EDWARDS A.L. Edwards Personal Pref rence S edul Revised manual Psy hological Corp New York 959

7 EDLUNDH, E & FUNDFORT S Th blood pressure repre ta ive population sample Acta med scand 183:293-305 1968

48 EDLUNDH E. & OHLSSON Rökning och arterosjukdom Bergsstudien. Läkartid 67 145-149 970

9 ELMFELDT D Rehabiliter patien er ed hjar infarkt Läkartid 67 65-167 1970

50 ELMFELDT WILHELMSEN L. TIBBLIN, G VEDIN J.A. WILHELMSSON C.-E. & BENGTSSON, C Th Myocardial Infarc on Regi te in Göteborg Swed In prepara io

5 ELMFELDT D WILHELMSEN L. TIBBLIN G VEDIN J A. WILHELMSSON -E. & BENGTSSON C Th Re Myocardial Infarc ion Clini in Gö borg, Swed In prepara i n.

52 ENTMACHER, & MARKS H.F Di in 1964 A world urve Diabete 14:212 223, 1965

53 EPSTEIN F.H The ep demi logy of oronary heart disease A review J chron.Di 18 735-77 1965

54 EPSTEIN F.H Hypergl cemia A risk facto in coronary heart diseas irc lation 36 609-6 9 1967

55 EPSTEIN F H, Some es of especitive hs rvations i th Tecumseh Community Heal S udy Proc Roy.Soc.Med 60 56-60 967

56 EPSTEIN F.H al pl risk facto and th pred ion f coronary eart disease J med.As Ga 60 15-38 971

57 EPSTEIN .H OSTRANDER J L. JOHNSON B AYER W KAYNER, E KELLER J.B & FRANCIS J T Epidemiological ud cardiovascular diseas in a tal communi Tecumseh Michigan Ann rn.Med 62 1 70- 187 965

58 EVANS J G. ROSE G Hypert sion Bri .med J ii, 77 37-42 197

59 FEJFAR Z Phi fac rs ackson eart diseas Acta cardi l Suppl 5 7 35 1972

60 FLECKER A PICHT F Marital status of coronary Inpiea Pos yrgr h cd med blik på p-pillers som ar malig ne alegi ahia Ugeskr la g 132:2401-2402 970

6 FODOR J he octoral and serum gl te Osteta g. 1 ad 11 68 31 69 Per ab J urnal 26-33, 969

62 FOPPIERAS O & LINDEBLÅD Th coronary pa A 1 14 ns linary i ve igs ion of Middle-aged fem nt eal J Ty eo he 31 3- 69 950

63 FRATLY P J & AMATOP E ... rel meas ... Acta 21 255-263, 196.
64 FRIBERG L, CEDERLÖF R, LUNDMAN T & OLSSON H ... tality ... Stud on ... Swedi ... Twin Regi try ... 21 508-513 1970
65 FRIL AR P & ACKERMAN R F ... blood ... cardio- ... blood ... ary art ... Ann 69 1286 1296 1959
66 FURMAN R L, ... (...) pp 02-404 Washington D C 1965
FYFE, T BAIRD R J COCHRAN K,M & BOOTH, E,M Plasma-lipid change after ... cardial ... 2 997 1001 197
68 GARCIA, P J GORDON T KANNEL, P R & KANNEL, W B Morbidity and ... tality ... general population ... 33 ... follow-up experience ... Diab ... 19 ... 1 375 1970
69 GR ... A, LARSSON T & ... J Diabete in Swed ... idemiolog cal ... and death certificate Acta med scand Suppl 77 1967
70 ... Coronary heart disease ... 1959-1961 ... mortality ... forlaget Oslo 1967
71 ... LIPID ... & lipop ... cardial ... compared with ... population sampl Cl ... 6 709-716 1972
72 ... & ... HANSEN ... 50-... Ugeskr Laeg 130:1... 1969
73 ... L, & ... populat on ... Ugeskr Laeg 33 1302 306 1971
74 HAYG ... LLOYD ... & PLAYFORD ... adrenal ... J ... Lond 178 ... 965
75 HALES ... & RANDLE P J Immunoassay ... -antibody precipitate Biochem J 88 ... 963
76 HALLBERG L, ... & ... blood ... popula ... 5 75 56 966
77 HALLER L, & ... lipid and ... plasma 50 ... 967
78 ... Acta ... 973
79 ... & STEIN ... 942
...

Proceedings of the Council for High Blood Pressure Research (ed. F R. Skelton) pp 53-81 American Heart Association, New York 1969

93. KANNEL, W B & CASTELLI, W.P The Framingham Study of coronary disease in women. Med Tms (N Y) 100 173-195 1972

94 KANNEL, W B CASTELLI, W P GORDON T & McNAMARA, P.M Serum cholesterol, lipoproteins and the risk of coronary heart disease The Framingham Study Ann Int Med 74 1 12 1971

95 KANNEL, W B CASTELLI, W.P & McNAMARA, P.M The coronary profile 12 year follow-up in the Framingham Study J occup Med 9 611-619 1967

96 KANNEL, W B CASTELLI, W.P & McNAMARA P.M. Cigarette smoking and risk of coronary heart disease Epidemiologic clues to pathogenesis The Framingham Study Nat Cancer Inst Monogr 28 9-20 1968

97 KANNEL, W B DAWBER T R KAGAN A. REVOTSKIE, N & STOKES J Factors of risk in the development of coronary heart disease six year follow-up experience The Framingham Study Ann Int Med 55 33-50 1961

98 KANNEL, W B & FEINLEIB M Natural history of angina pectoris in the Framingham Study Prognosis and survival Am J Card 29 154-163, 1972

99 KANNEL, W B GORDON T & SCHWARTZ, M.J Systolic versus diastolic blood pressure and risk of coronary heart disease The Framingham Study Am J Cardiol 27 335-346 1971

100 KANNEL, W B LeBAUER E.J DAWBER T.R & McNAMARA, P M Relation of body weight to development of coronary heart disease The Framingham Study Circulation 35 734-744 1967

101 KANNEL, W B McNAMARA P.M FEINLEIB M & DAWBER T R. The unrecognized myocardial infarction Fourteen-year follow-up experience in the Framingham Study Geriatrics 25 75-87 1970

102 KANNEL, W B SCHWARTZ M.J & McNAMARA, P M Blood pressure and risk of coronary heart disease the Framingham Study [illegible] 56 3-[illegible] 1969

103 KERSHBAUM A. BELLET S [illegible] & FEINBERG L.J Effect of cigarette smoking and nicotine on [illegible] free fatty acids Based on studies in the human subject and the experimental animal Circulation Res 9[illegible]631-638 1961

104 KEYS A. [illegible] of research on the epidemiology of coronary heart disease Jap Circulat J (T) 31:1669-1683, 1968

105. KIPNIS D.M Insulin secretion in normal and diabetic individuals Adv in Int.Med 16 103-134 1970

106 [illegible] [illegible] C T [illegible] C & [illegible] B Effect of diuretic therapy on glucose tolerance in hypertensive patients Lancet 986-990, 1971

107 Leading article Cigarette smoking and heart disease Brit.med J 2:1404-1405 1966

108 Leading artical [illegible] og [illegible] Farm [illegible] komplikasjoner Tidsskr Norske Laegeforen 91 1413-14 1971

109 LEHMAN, E.W Social class and coronary heart disease: a sociological assessment of the medical literature J chron.D 20 381-391 1967

110 LEVI P The effect of [illegible] on lowering and survival of myocardial infarction [illegible] clinical trial Acta med scand suppl 466 1966

111 LEVIN E. & [illegible] [illegible] blood lipemia [illegible] gly on [illegible] [illegible] J clin. [illegible] 45:3016-3026 1966

112 LEVINE J B [illegible] An automated determination of serum total [illegible] Clin. [illegible] 10:381-[illegible] 1964

113 LILJEFORS I Coronary heart disease in male twins Hereditary and environmental factors in concordant and discordant pairs Acta med scand suppl 511 1970

114 LITTLE [illegible] [illegible] & [illegible] Fórsøk med [illegible] Tids Norske Laegeforen 76 [illegible] 1956

115 LOPTLAND J [illegible] determination of triglyceride serum [illegible] 91:393-400 1966

116 LU [illegible] [illegible] coronary [illegible] experimental therapy [illegible] [illegible] 5-433 1969

117 [illegible] [illegible] heart disease and [illegible] Acta med scand [illegible] 55 1966

118 [illegible] [illegible] [illegible] [illegible] Ty [illegible] plasma lipids [illegible] myocardial infarction The respective effect of age [illegible] Europ J [illegible] 265-270 1971

119 MAR [illegible] J [illegible] O'[illegible], [illegible] and [illegible] ratios and [illegible] of coronary [illegible] Am Card 26 33-37 1970

120 MAR [illegible] Q [illegible] ALTMAN [illegible] D [illegible] low-density lipoprotein [illegible] myocardial infarction and death [illegible] ischemic heart disease [illegible]

121 MAF MITON J MOORE F J HOFFIM C E FURMA, O P & EIFLER J Clinical studi of long term anticoagulant therapy in with myocardial infarction Proc Soc Exp Biol (N Y) 110 400-409 1962

122 P THIL, A E Analyzing qualitative data P tree & Co London 1961

123 Medical Research Council Committee on the Aetiology of Chronic Bronchitis Definition and classification of chronic bronchitis for clinical and epidemiological purposes Lancet 1 775-779 1965

124 PIALL, W E heredity and hypertension Practitioner 207 20-27 1971

125 PIALL, W E, DEL CAMPO E FODOR J MATA PHOSE J R, PUTZ L, STAMLER E L, & PHAS A T Longitudinal study of heart disease in Jamaican rural population I Prevalence with special reference to ECG findings Bull Wld Hlth Org 46 429-441 1972

126 MOLLER P Lat Diabetes al Risikofaktor bei Myokardinfarkt Z.ges inn Med 23 89-90 1968

127 MORRIS J N Occupation and coronary heart disease Arch.int Med 104 903-907 1959

128 MATTILA M & MATTILA, M Myocardial infarction and coronary thrombosis in women under the age of 45 years in Finland Beitr path.Anat 1 1 233-237 1970

129 MULCAHY R HICKEY N & MAURER J F The role of cigarette smoking in the causation of coronary heart disease Circulation 22 165-17 1967

130 MULCAHY R HICKEY N, & MAURER B & C Coronary heart disease in women Study of risk factors in 100 patients less than 60 years of age Circulation 36 577 586 1967

131 MURRAY H.A Explorations in personality Oxford University Press New York 1938

132 NIKKILA E.A PIETTINEN T A, VIRKKUNEN M P & PELKONEN R, Plasma-insulin in coronary heart disease Response to oral and intravenous glucose and tolbutamide Lancet 2 508 511 1965

133. NIESSLER B STEINER O & THORELL, J Faktoren des Todes bei Herzinfarkt Lakartid 69 5225-5227 1972

134 NOVAK, E P & WILLIAMS T J Autopsy comparison of cardiovascular changes in castrated and normal women Am J Obstet Gyn 80 863-873 1960

135 NYE E,R Coronary heart disease and lipid in hypertension J.A Trop 1 P 61 8-25 1966

136 NYQUIST O & E Complications of myocardial infarction Acta med scand Suppl 534 1972

137 OLIVER M F Coronary artery disease in women In Pathogenesis and treatment of occlusive vascular disease (ed L. McDonald) pp 124-132 Pitman Medical Publishing Co Ltd London 1960

138 OLIVER M F Ischaemic heart disease in young women Brit Heart J 27 305 1965

139 OLIVER M F Presymptomatic diagnosis of ischaemic heart disease In Proceedings of the Fifth World Congress of Cardiology Symposia New Delhi 1966 pp 377-390 Brussell

140 OLIVER M F Oral contraceptives and myocardial infarction Brit med J 2 210-213 1970

141 OLIVER M F & BOYD G S Influence of reduction of serum lipids on prognosis of coronary heart disease A five year study using oestrogen Lancet 2 499-505 1961

142 OLIVER M F Sex and coronary heart disease Brit Heart J 30 1 5-150 1968

OSTFELD A,M LEBOVITS B Z SHEKELLE, R B & PAUL, O A prospective study of the relationship between personality and coronary heart disease J chron Dis 17 265-276 1964

OSTRANDER J L.D FRANCIS J T HAYNER N S KJELSBERG M,O & EPSTEIN F,H The relationship of cardiovascular disease to hyperglycemia Ann.int Med 62 1188-1198 1965

OSTRANDER E,F GAP C A, HALL, G & KING T M, The incidence of coronary arteriosclerosis in premenopausal women ... Am J Obst Gy 99 55-162 1967

146 OSTRANDER E F GAPP C A SILER J, S L, & KING T The relationship of pregnancy to coronary artery atherosclerosis Am J Obst Gyn 97 287 091 1967

147 OTHER CH L & SLA J Lipid ... and feral survivors of myocardial infarction ... Lancet 1 393-399 1972

EAP I I ... myocardial infarction ... 7 648-650 1971

149 HITER ... C B ... myocardial infarction Lancet 1 1965

... effects ... after ... 1 7 1 26 8 2 18 5 1971

15 ... A.A. ... L, I ... myocardial infarction J ... 9 24-535 1971

152 ... B ... and ... glucose ... of myocardial infarction J 26 3 1966

153. ... B JAFF I ... MASLANKA, J.P & PEED E Coronal function ... of ... 237 25 1963

... heart disease ... study ...

155 ROSE, G.A : The diagnosi of ischaemic heart pain and internal ten claudication in fi ld survey Bull Wld Hlth Org 27 645-658 1962

156. ROSENMAN R.F & FRIEDMAN, M : Association of specifi behavior pat ern in men with blood and cardiovascular findings Circ la ion 24 1173-1184 1961

157 ROSENMAN, R.F FRIEDMAN M STRAUS R. JENKINS C.D ZYZANSKI, S J & WURM M Coronary heart disease in th west rn collabora ive group tudy A f llow-up xperience of 4 ½ years J chron.Dis 23:173-190 1970

158 RYAN W G, SCHWARTZ T G, & SCHWARTZ T B : Intravenous glucose tolerance as in an industrial population, Cont l subjects wi oronary heart diseas and relative of diab tic Diabetes 6 171-174 1967

159 S ientifi tabl 7 h ed (ed K. Diem & C Lentner) p.38 Ciba-Geigy Basl 1971

160 Scientif tabl 7th ed. (ed K. Diem & C Lentne) p 711 Ciba-Geigy Basl 971

161 SELTZER C C An evaluation of the ffec of smoking on oronary heart disease I. Epidemi logical evidence J ame .med.As 203:193-200 1968

162 SHAPIRO S WEINBLATT E, FRANK C W & SAGER, R.T The H I,P Stud of i ncidence and p ognosi of oronary heart disease Pr liminary findings on incidence of myocardial infarction and angina. J chr n.Di 18 527-558 1965

163 SHAPIRO S WEINBLATT E FRANK, C & SAGER, R. : Incid nce f oronary heart disease i popu-la ion i sured f medical care (HIP) Myocardial infarc on angina pectoris and possibl myocardial infar tion Am J publ Hl h 59 uppl 2 1 101 1969

164 SPIEKERMAN, R E ORTFELD A M & PAVL, O. Social status and incidence of cor nary heart di ease J chron Dis 22 38 394 1969

165. SPROUTT S P Occurrence of myocardial nfarction in wome 50 year of age and younge Report pe iens S al Hosp tal S nai Hosp J (Balti ore) 9:206-211 1960

66 SIEGEL, S Nonparametri stati f th behavioral sci nce McGra Hill Book Comp Ne Y rk 956

67 STAMLER J W Th ta us of risk fac ors and coronary eart di eas J Pre Di 22 515-532 970

168 STAMLER J Atr rosal roti ronary eart disease ti logy and pathogene ronary ri k factors In Lec ur on pr ve tive card logy pp 07-162 Grune & S ra ton Inc Ne Y and London 1967

69 STAMLER, J Ep dem ology of oronary eart disease Med Clin.N.Am 57 5-46 1973.

170 STAMLER J PICK KAT L.N PICK KAPLAN, B.M BERKSON D.M & CENTURY D Effectiveness of st ogens f th rapy of myocardial nf rction i middle-age men J ame med.As 183:632-638 1963

71 ta onal yearbook of Go berg 1968 1 68 G6 bo g Off ce of Sta i i Go berg 1968

172 tat tional yearbook of Go berg 969 69 Go berg Off ce f Sta stics, Goteborg 969

73 Sta al yearbook f Go erg 1970 vol 70 Got borg Office of Sta i tics Got borg 970

174 STERNBY N H ae in defined population An autopsy survey in Malmö Sweden Acta path.microbiol scand uppl 194 1968

175 T eal onsequences f smoking S Departme of health, Education and W lfare Publi Health Servi Pu l on No 1696 Was ington, D C 968

76 THEORELL, T & RAHE, R.H. ynchronic factors and myocardial nfarction I. An inpatien tudy in Swede J psychosom R 5 25-3 971

77 rans Admini ra on co-opera ve Urological R search Group Treatmen and urvival of pa en wi cance of pro tate Surg.Gyne Obst 24 1011-1017 1967

78 THOMAS C B On igar te smoking oronary heart disease and the gene Bull Johns Hopk. med 22 69-76 968

79 THORELL, J I ERSSON B STERNBY early ns lin response to in rave ous glu injection J la in.Med In pre

180 TIBBLIN, G BENGTSSON C BLOHMÉ, G COSTAFSON, A. Serum chol terol and serum triglyc rid aged wome T udy of Wome i Gote org 1968-1969 In reparation

1 TIBBLIN G. ep la on tudy of 50- ear-old men. An analy of the non-parti pa ts gr up Acta med scand 78 53-459 965

TIBBLIN, Hig lood pre ur aged 50 popula on tudy of men orn i 1913. Acta med 70 967

83 TIBBLIN G Angina pe ori ro men for lopp och prognos In: Angina pectori (Ed E. Varnauskas & L. rk) A lindgr & Go 969

184 TIBBLIN G H fac oronary eart disease Am Card 15- 30 1970

18 TIBBLIN G factors for de lop ng ocardial infarction and the diseas Th op tors i 9 3 ed I re ve card logy (eds G Tib lin, A. Key L. Werkö) pp 33-42 Almqvi & Wiksell Forlags AB Stock lm 972

186 TIBBLIN G & BENGTSSON C De esti and t esta f typ t ies i annual y Hilta k men Fd Cmart 7 160-169 1969
187 TIBBLIN G & CRAMER K Serum lipid during th eours f an t myocardial i farc i and on year afterwards Act ed sand 174 451-455 1963.
188 TRACY R E & diff re se i orary disease two app ai g i va J Chro Di 19:1245-1251 1966
189 TZAGOURNIS M SEIDENSTICKER, J F & HAMWI, G J Serum insulin oart hydrat and lipid te rmali ti i pati ts with premetur coronary heart disease Ann int rn Med 67 2-47 1967
190 UTIAN W H, Ch l arol coronary heart di eas and oe trage Sth Af med J 5 359-361 1971
191 VEDIN J.A WILHELMSSON C E, BOLANDER, A -M & WERKO L, Mortality trend in Swed 1951 1968 with special f rence to cardi vasc lar cas f death, Acta med scand S ppl 515 1970
192 VILLINGER, W & LEYDEN S Neue Ergebni se de epid i logi F rschung bei d ischami he Herz krankung I Retr spektive pidemi logische Be bacht ng d Alter lhesume d Infarktpati t Z Freisl F rsch 35 738-742 1966
193 WAHLBERG F I trave ous gluc t l rance i myocardial infarcti n, angina pect ri and i rmal te 1 ndica ies Act ed scand Suppl 53 1966
194 WAILER, E B KIVNINIIS D VAN DER BROEK F SEGAL, B L & LIKOFF W My cardial i farc ion and aral co raceptive age t Am J Cardi 1 28 96-101 1971
195 WEAVER J A BEATIA, S F BOYLE, D HADDEN D R, & MONTGOMERY D A.D Cardi vas ular te f newly dia ed diabeti es Bri med J 1 783-786 1970
196 WILCOKS T A COMPSTON G N CULLEN K.J CURNOW D H McCALL, M G & STENHOUSE N S Th p e-valence f coronary heart disease and as ociated factors an A tralian rural annual Am J Epidemi l 89:152 556 1969
197 WESTLUND K Furth bservat ns on th incid nce f myocardial inf rc ion in Osl J Osl Cy H sp 15 201 231 196
198 WESTLUND K Myocardial infarction i Osl 1967 1969 Incid and case f ality J Osl Cy H sp 22 77 108 1972
199 WESTLUND K & NICOLAYSEN, R T n-yea tal y and morbidi y related ru l t rol A f llow-up f 3 751 me aged 40-49 Scand J Cl Lab I t S ppl 127 1972
200 WIKLAND B Medicall re rded f tal case of ischaemic heart disease i d fi d pop la i Acta ed scand Suppl 524 971
201 WILHELMSEN L, Th my cardi l farc ion li i in G be g ganiza ion and prelimi ry l P H Dak J ar l 5-54 1969
202 WILHELMSEN L, Serum l rol and lood p bef and re sem af myocard l f rc ion I p para ion
203 WILHELMSEN L, BJURE J EKSTRÖM JODAL, B AURELL, M GRIMBY G & TIBBLIN G lon record o-grup i pa ra duri g ard re ae represe ive pop l i sampl Th udy f Me Born 1913 I prepara on
204 WILHELMSEN L & TIBBLIN G Ph sml ana i y and ri k f ocardial afar ion T M Born 913 ud In C onary Hear di ease nd phy cal f tn (ed O A, Larse & R O Male rg) pp 251 255 M k gaard ope hage 1971
205 WILHELMSEN L, TIBBLIN G WERKO L, A primary p ve nd Go burg Swed Pre Med 53- 60 972
206 WILHELMSEN L, ELYL, F & TIBBLIN G M l varia analy of ri k factors f co onary hear disease me aged 50 years in Go berg, Swede irc la l p
207 WISE K, & RAOLF hard fark i ns re re d Praxe Med Klin, 67 364 372 972
208 WINT LOT IN J & FILATE, Age read f rtal f co onary art ry d eas ee d ex ace ex p edve ire p rr f thos aff ed Ame Hear J, 67 48 -488 964
209 ISY LOTEI J T FELTER M A, LILIENFELD A.M Oc urrence f pregr ncy t rt d ar ial embopa among ome wi ronary ar ry disease preli inary udy J Chron Dis 7 273- 958

KUNGSBACKA
ELANDERS BOKTRYCKERI AKTIEBOLAG
1973

Acta Medica Scandinavica

Supplementum 550

Beta-Adrenergic Blockade in Essential Hypertension

Effects of propranolol on hemodynamic parameters and plasma renin activity

By Lennart Hansson

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each annually) include free supplements to the current volumes.

Subscription Rates

Per annum = two volumes.
In Denmark, Finland, Iceland, Norway Sweden and the Netherlands: Sw cr 170 incl. postage.
Other countries: Sw cr 200, incl postage

Chief Editor

Professor Jan G Waldenström MD
Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm Sweden

Editorial Office

Acta Medica Scandinavica
Kungsgatan 54
S-111 35 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Kungsholmsstrand 175
S-112 48 Stockholm, Sweden

Subscription and Distribution

The Almqvist & Wiksell
Periodical Company
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1 Sweden

Printers

Almqvist & Wiksell Inform
industri AB
S- sala, Sweden

From the Department of Internal Medicine I Sahlgren s Hospital,
University of Göteborg, Sweden

Beta-Adrenergic Blockade in Essential Hypertension

Effects of propranolol on hemodynamic parameters and plasma renin activity

By
LENNART HANSSON

GÖTEBORG 1973

This study is based on the following papers.

I Hansson, L. & Zweifler A.J The effect of propranolol on plasma renin activity and blood pressure in essential hypertension.

II Hansson, L., Zweifler A.J Julius, S & Hunyor S.N Hemodynamic effects of acute and chronic beta-adrenergic blockade in essential hypertension.

III Hansson, L & Zweifler A.J Correlation of the degree of beta-adrenergic blockade to plasma propranolol concentrations in the treatment of hypertension. The clinical usefulness of the nitroglycerine test.

IV Hansson, L Zweifler A.J Julius, S & Ellis, C.N., Propranolol therapy in essential hypertension. Observations on predictability

Printed in Sweden
Falkenbergs Tryckeri AB
Falkenberg 1973

CONTENTS

ABBREVIATIONS AND NOMENCLATURE

BSA = Body Surface Area
HR = Heart Rate
LVH = Left Ventricular Hypertrophy
MAP = Mean Arterial Pressure
n.s. = Not significant
PRA = Plasma Renin Activity
Q = Cardiac Output
Qi = Cardiac Index
SV = Stroke Volume
SVI = Stroke Volume Index
TPR = Total Peripheral Resistance
TPRi = Total Peripheral Resistance Index

The somewhat controversial expression "essential hypertension" has been used throughout this paper to indicate primary hypertension, i.e. extensive clinical and laboratory studies have not revealed the etiology of the high blood pressure. Furthermore, "propranolol" is used for dl-propranolol chloride.

INTRODUCTION

The present study is concerned with the effects of acute and prolonged beta-adrenergic blockade in essential hypertension. Special interest has been devoted to hemodynamic alterations and changes of plasma renin activity seen after acute intravenous administration of propranolol and following four weeks of oral treatment.

Hemodynamics in essential hypertension

It is generally agreed that total peripheral vascular resistance at rest is elevated in patients with essential hypertension while cardiac output is normal [19, 37, 80]. Early phases, e.g. borderline hypertension constitute exceptions as cardiac output may be increased as well [30].

The underlying mechanism by which peripheral vascular resistance is elevated is not fully understood. A change of the wall/lumen ratio in the resistance vessels, e.g. due to smooth muscle hypertrophy [80] or water logging due to increased sodium content of the vessel wall [73] is conceivable.

Increased vasoconstrictor nerve activity has not been the considered an important mechanism for maintainance of the elevated resistance and recent microneurographic studies have not revealed higher activity in effecent sympathetic nerves in hypertensive patients than in normals [78].

Elevated levels of plasma catecholamines have recently been reported in essential hypertension [10, 4] which may seem surprising in view of the finding that urinary excretion of norepinephrine is not increased [5].

Another factor of importance for vascular resistance is blood viscosity. It has been reported that patients with essential hypertension have increased blood viscosity [70] but it should be pointed out that in vitro measurement of this parameter may be an unreliable indicator of in vivo viscosity [11]. Others consider viscosity to be within normal limits in essential hypertension [47].

In summary elevation of blood pressure in established essential hypertension is an effect of inappropriately elevated total peripheral resistance in relation to cardiac output.

Plasma renin activity

A humoral pressor substance of renal origin was first described – and given the name renin – by Tigerstedt and Bergman in 1898 [72]. The classic observation in 1934 by Goldblatt et al. that renal ischemia produced hypertension [22] caused a renewal of the interest in renin. Later the renin-angiotensin system was described [4, 46, 60, 62, 74]. Aldosterone was isolated [58] and the relation between renin/angiotensin and aldosterone was discovered [33, 34, 43].

Initiated by these fundamental studies, a number of investigations have increased our knowledge of renin, angiotensin and aldosterone in various kinds of hypertension.

As a rule, plasma renin activity is not elevated in benign essential hypertension [16, 26, 79] and aldosterone secretion is normal [16, 36]. Low plasma renin activity is seen in approximately 25 % of patients with benign essential hypertension [8, 29]. On the other hand, elevated renin levels and increased excretion of aldosterone are prevalent findings in malignant hypertension [33, 34].

In 1960 Laragh suggested that the vascular damage in malignant hypertension was related to elevated levels of angiotensin [33, 35]. Support for this hypothesis is given by results from animal research in which excess renin/

angiotensin has been shown to cause vascular lesions of the same appearance as those in malignant hypertension [6, 21, 22, 30]

Recently it has been suggested that low plasma renin in patients with essential hyper tension is associated with a reduced risk of development of cardiovascular complications such as stroke and myocardial infarction [6]. A positive relationship has been reported also between plasma renin and the severity of hypertensive retinopathy [79].

If these observations hold true it would seem to be preferable to employ therapy that not only lowers blood pressure but reduces renin as well in the care of hypertensive patients.

A number of antihypertensive drugs cur rently in use have been studied with regard to their effect on plasma renin activity. Elevation of plasma renin has been reported as a result of treatment with thiazide diuretics [2, 84], hydralazine [30], diazoxide [32] and sodium nitro prusside [31].

On the other hand, drugs with adrenergic inhibitory effects may cause reductions of plasma renin activity. This has been shown e.g. for alpha-methyldopa, clonidine [44], propranolol [4, 40, 80], while there are different opinions regarding the effect of phentolamine [40, 80].

In summary: Plasma renin activity is usually elevated in malignant hypertension and normal or low in benign essential hypertension. The observation that low plasma renin may be associated with a reduced risk of car diovascular complications deserves further investigation. Implications regarding the possibilities of a prophylactic effect of plasma renin lowering therapy are not justified at present.

Beta-adrenergic blockers in hyper tension

Antihypertensive effect of beta-adrenergic blocking drugs was first reported with the use of pronethalol [48, 5], a drug that has not been explorted in view of its tumor producing action in mice [48].

The first report of antihypertensive action of currently used beta-adrenergic blocking agents was published in 1964 by Prichard and Gillam [49]. They reported effective reduction of blood pressure following oral administration of propranolol. Later they extended their studies to larger numbers of patients [50, 51] and concluded that propranolol was an equally potent antihypertensive agent as e.g. guanethidine with the advantage of not producing postural or exercise hypotension [51]. Subsequently many positive reports have been published regarding the usefulness of propranolol in hy pertension [20, 25, 61], although occasionally no effect has been observed [27]. Other beta adrenergic blocking agents have also proved to be useful in hypertension, e.g. alprenolol [71], oxprenolol [12] and practolol [82].

From a theoretical point of view the antihy pertensive action of propranolol may seem paradoxical considering its predominantly cardiac effects [30]. Furthermore, as acute intravenous administration of propranolol does not reduce blood pressure in patients with normal rhythm [75], it has been difficult to understand the mode of action of the drug.

Several suggestions have been offered in an attempt to explain how blood pressure is reduced by propranolol. Reduction of cardiac output does not by itself lower blood pressure due to a compensatory increase of peripheral vascular resistance [75]. Resetting of baroreceptors has been suggested in response to reduction of the cardiac component of transient rises of blood pressure [50, 51].

However, so far this hypothesis has not been confirmed.

In animal research it has been shown that reduction of blood pressure may result from the local anesthetic effect of propranolol causing adrenergic nerve blockade [13]. Hypotension could be produced in rabbits also by the dextroisomer of propranolol which retains the

local anesthetic effect of 1-propranolol but has less than one hundredth of the beta-adrenergic blocking effect [1] However the anti-hypertensive effect of d-propranolol was negligible when given to 35 hypertensive patients whereas dl-propranolol proved to be effective [77]

The importance of this mechanism in man therefore remains doubtful.

Plasma volume contraction is not a reproducible finding during chronic propranolol therapy [66,67] and can not be responsible for the reduction of arterial pressure

Finally adaptation of peripheral vascular resistance in response to long-term reduction of cardiac output [68] and relation of blood pressure reduction to suppression of plasma renin activity [6] have been suggested Both possibilities will be commented upon in the discussion as part of the present study was devoted to these problems.

In summary: Beta-adrenergic blockade has been shown to reduce blood pressure effectively in hypertensive patients without causing postural hypotension or reduction of blood pressure during exercise. There are controversial opinions regarding its mode of action and this will be discussed further in this paper

angiotensin has been shown to cause vascular lesions of the same appearance as those in malignant hypertension [9, 21, 22, 38]

Recently it has been suggested that low plasma renin in patients with essential hypertension is associated with a reduced risk of development of cardiovascular complications such as stroke and myocardial infarction [8]. A positive relationship has been reported also between plasma renin and the severity of hypertensive retinopathy [79].

If these observations hold true it would seem to be preferable to employ therapy that not only lowers blood pressure but reduces renin as well in the care of hypertensive patients.

A number of antihypertensive drugs currently in use have been studied with regard to their effect on plasma renin activity. Elevation of plasma renin has been reported as a result of treatment with thiazide diuretics [2, 44] hydralazine [38] diazoxide [32] and sodium nitro prusside [3

On the other hand, drugs with adrenergic inhibitory effects may cause reductions of plasma renin activity. This has been shown e.g. for alpha-methyldopa) clonidine [44] propranolol [8, 40, 80] while there are different opinions regarding the effect of phentolamine [40, 80]

In summary: Plasma renin activity is usually elevated in malignant hypertension and normal or low in benign essential hypertension. The observation that low plasma renin may be associated with a reduced risk of cardiovascular complications deserves further investigation. Implications regarding the possibilities of a prophylactic effect of plasma renin lowering therapy are not justified at present.

Beta-adrenergic blockers in hypertension

Antihypertensive effect of beta-[illegible] blocking drugs was first report[illegible] [illegible] of pronethalol [48] [illegible] a drug that ha[illegible] exploited in view of its tumor producing action in mice [48].

The first report of antihypertensive action of currently used beta-adrenergic blocking agents was published in 1964 by Prichard and Gillam [49]. They reported effective reduction of blood pressure following oral administration of propranolol. Later they extended their studies to larger numbers of patients [50, 81] and concluded that propranolol was an equally potent antihypertensive agent as e.g. guanethidine with the advantage of not producing postural or exercise hypotension [61]. Subsequently many positive reports have been published regarding the usefulness of propranolol in hypertension [30, 26, 81] although occasionally no effect has been observed [27]. Other beta-adrenergic blocking agents have also proved to be useful in hypertension, e.g. alprenolol [71] oxprenolol [12] and practolol [53]

From a theoretical point of view the antihypertensive action of propranolol may seem paradoxical considering its predominantly cardiac effects [30]. Furthermore as acute intravenous administration of propranolol does not reduce blood pressure in patients with normal rhythm [76] it has been difficult to understand the mode of action of the drug.

Several suggestions have been offered in an attempt to explain how blood pressure is reduced by propranolol. Reduction of cardiac output does not by itself lower blood pressure due to a compensatory increase of peripheral vascular resistance [76]. Resetting of baroreceptors has been suggested in response to reduction of the cardiac component of transient rises of blood pressure [60, 81].

However, so far this hypothesis has not been confirmed.

In animal r[illegible]search it has been shown that r[illegible]du[illegible]t[illegible]on [illegible] blood pressure may result from th[illegible] [illegible]l anesthet[illegible] effect of propranolol caus[illegible] [illegible] adr[illegible]nergic nerve blockade [13]. Hypoten[illegible] [illegible] [illegible]ould be produced in rabbits also by the [illegible] [illegible] [illegible] of propranolol which retains the

as the study was started with a four week placebo period. To further reduce risks during the placebo period all patients were instructed to contact the clinic should diastolic home blood pressure rise above 120 mm Hg at two successive measurements.

After being informed about all details of the study except the initial placebo period, both verbally and in writing, all patients gave their written consent to participate.

Material

Initially 19 patients were recruited to the study They had all been investigated for their hypertension at either the University of Michigan Medical Center or the Veterans Administration Hospital in Ann Arbor There were two early drop-outs. One patient showed a rise of diastolic home blood pressure to over 140 mm Hg already during the first week of placebo treatment and had to resume active therapy The second patient was normotensive (less than 150/90 mm Hg) at the end of the placebo period. In addition, his home blood pressure had been normal throughout the four weeks of placebo treatment.

Of the remaining 17 patients two refused the second hemodynamic study at the end of four weeks of propranolol therapy thereby reducing the number of patients who completed the study to 15 No patient was withdrawn during the study e.g. due to side-effects or unsuccessful catheterizations.

The diagnosis of essential hypertension was made after careful clinical evaluation (history and physical examination) laboratory studies including serum electrolytes, serum creatinine, urinary excretion of aldosterone, catecholamines or vanillomandelic acid, tests for proteinuria, urine sediment and rapid sequence intravenous pyelograms.

The average age was 45 years (range 26–66) and the average known duration of hypertension was 7 years (range 0–17) All patients had benign hypertension as judged by fundoscopic examination. Only one patient had left ventricular hypertrophy as estimated by chest

Table I **Patient Information.**

Patient	Age years	Race*	Duration of hypertension years	Eye** grounds	Serum creatinine mg/100 ml	Creatinine clearance 1/24 h	LVH*** EKG/ chest X-ray	Previous § therapy	Initial ‡ Blood Pressure mm Hg	Placebo ‡‡ Blood Pressure mm Hg
1 F.L.	50	C	10	I	0 9	180	–	A D	130/90	159/98
2 H.T	50	B	2	II	1 1	216	– –	D	142/112	150/110
3 L W	55	B	2	I	1 0	142	–	D	144/100	155/110
4 L.A.	53	C	7	I	1 0	125		D	125/88	180/120
5. T.D	66	C	13	I	1 2	88	–	R	194/97	169/86
6 D B	26	C	0	I	1 1	N A		–	140/91	165/95
7 R.R	40	C	17	II	1 6	162	–	G D	162/98	174/137
8 M E	27	B	8	0	1 1	177	–	D	121/92	134/95
9 M D	52	C	0	I	0.9	149			136/99	155/108
10 L.K	49	C	7	II	1 1	N A	+	A D	182/113	200/128
11 J.K	45	C	7	II	1 2	N A		A D	149/107	161/111
12 M P	44	C	9	I	1 0	155			152/96	177/107
13 G H	41	C	10	II	1 1	191	+ +	A D	179/123	184/105
14 A.P	43	C	0	II	1 0	150	–	D	143/98	140/96
15 F P	35	C	6	II	0 9	198			164/130	162/110

*C Caucasian B Black **Keith Wagener and Barker (1939) classification. ***Left ventricular hypertrophy §A Alpha-methyldopa, D Diuretics G Guanadrel and R Reserpine ‡ At admission to study measured in the recumbent position ‡‡ After 4 weeks of placebo N A Not available

X-ray and EKG while one patient fulfilled the EKG criteria only. One patient had impaired renal function as judged by serum creatinine (1.6 mg/100 ml) while the remainder had normal serum creatinine concentration.

Before admission to the study 11 patients were receiving antihypertensive therapy regularly. This consisted of diuretics in 5, alpha-methyldopa and diuretics in 4, reserpine in 1 and guanadrel and diuretics in 1. The remaining four patients were untreated although 3 of them had been treated earlier. For information on individual patients see Table I.

Methods

Clinic blood pressures

All out-patient visits were in a special research clinic, the Upjohn Center for Clinical Pharmacology at the University of Michigan Medical Center, Ann Arbor. Throughout the study the same two nurses recorded all blood pressures. Their correlation was checked frequently by simultaneous readings using a "Y" connection. A mercury manometer attached to a 14 cm wide cuff was used.

Readings were always made in the morning in a quiet airconditioned room. Recumbent pressures were measured after 15 minutes of rest and standing pressures were recorded 2 minutes after assumption of upright posture.

Recumbent and standing pulse rates were measured accordingly. Phase V (disappearance) of Korotkoff sounds was taken as the diastolic end point. With few exceptions the average of 3 readings was calculated.

Clinic blood pressure was recorded at the beginning of the study, after 4 weeks of placebo treatment and after 2 and 4 weeks of oral propranolol thera

Home blood pressures

All patients were carefully instructed how to measure blood ressure and their accuracy was checked by the nurses using a "Y" connection. They were then provided with specially designed home blood pressure sphygomanometers (Propper) and instructed to record home blood pressure mornings and evenings in the recumbent and standing positions. Recumbent pulse rate was also recorded. Daily measurements of home blood pressure and pulse rate were continued throughout the study. Special sheets were used for recording of these parameters and side-effects or comments. Home blood pressures were consistently lower than corresponding recordings in the clinic. Patient No. 5 was an exception in this respect as his recordings at home were higher than in the clinic. Clinic blood pressures were significantly correlated to home blood pressure both after 4 weeks of placebo (Fig. 1) and after 4 weeks of propranolol (Fig. 2) independently of whether morning or evening home blood pressures were used.

Hospital investigations

After 4 weeks of placebo treatment all patients were hospitalized during 4 days in a metabolic research ward – the Clinical Research Unit – for baseline laboratory studies. Placebo treatment was continued during the stay in hospital. Studies of 12-hour urinary excretion of catecholamines were made twice with collections starting at 8 p.m. A 24-hour endogenous creatinine clearance was made and, ending on the morning of the fourth day, studies of 24-hour urinary excretion of aldosterone, sodium and potassium.

A simple no-added-salt diet was introduced and the patients were instructed repeatedly by a dietician regarding the continuation of a similar diet following discharge.

An extensive number of blood (serum) and urine tests were made repeatedly during the study. Ekg and chest x-ray were performed initially and following the placebo and propanolol periods.

Hemodynamic studies

Cardiac output determinations

The hemodynamic studies were performed twice in every patient. The initial study was made after 4 weeks of placebo treatment and the second was performed after the propranolol period. The hemodynamic studies were made in the morning with the patients fasting.

An 18-gauge teflon catheter was inserted into the left brachial artery and a polyethylene catheter (PE 50) was introduced percutaneously into the left antecubital vein and propagated to a central vein. Systemic arterial pressure was measured by a Statham strain gauge transducer (P 23 G) kept at a level 5 cm below the sternum.

Cardiac output was determined by the indicator dilution technique [42] using indocyanine green (Cardio-Green) and a Gilford densitometer Direct dye calibration was performed after each prodedure by the addition of 5 10 and 15 μl of Cardio-Green (3 mg/ml) to 10 ml aliquots of blood which were then drawn through the densitometer

Respiration was monitored continuously by means of a bellows attached around the chest and connected to a strain gauge by a rubber tube.

Standard lead I of the EKG together with intra-arterial blood pressure, respiration and dye curves were recorded on a Gilson poly graph.

Stroke volume (SV) was calculated by dividing cardiac output (Q) by heart rate (HR) Total peripheral resistance (TPR) was obtained by dividing mean arterial pressure (MAP) by Q and was expressed in arbitrary units (u)

Body surface area (BSA) was calculated from the formula. $BSA = W^{0.425} \times H^{0.725} \times 71.84$ (DuBois and DuBois) where W = weight in kg and H = height in cm.

By compensating for BSA the corresponding indices QI, TPRI and SVI were calculated

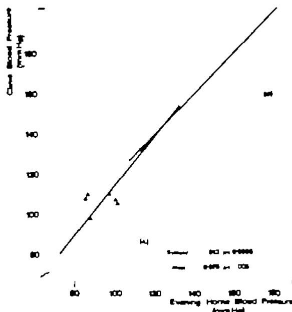


Fig 1
Correlations between recumbent clinic blood pressure at the end of 4 weeks of placebo treatment and average recumbent evening home blood pressure during the last week of placebo Readings in patient No 5 are within brackets and have not been included in the calculations (see text)

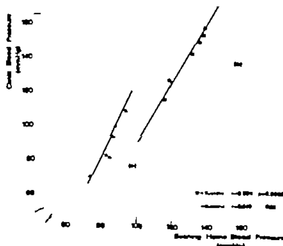


Fig 2. Correlations between recumbent clinic blood pressure at the end of 4 weeks of propranolol treatment and average recumbent evening home blood pressure during the last week of propranolol. Readings in patient No 5 are within brackets and have not been included in the calculations (see text)

X ray and EKG while one patient fullfilled the EKG criteria only One patient had impaired renal function as judged by serum creatinine (1 6 mg/100 ml) while the remainder had normal serum creatinine concentration

Before admission to the study 11 patients were receiving antihypertensive therapy regularly This consisted of diuretics in 5 alpha methyldopa and diuretics in 4 reserpine in 1 and guanadrel and diuretics in 1 The remaining four patients were untreated although 3 of them had been treated earlier For information on individual patients see Table I

Methods

Clinic blood pressures

All out-patient visits were in a special research clinic, the Upjohn Center for Clinical Pharmacology at the University of Michigan Medical Center Ann Arbor Throughout the study the same two nurses recorded all blood pressures. Their correlation was checked frequently by simultaneous readings using a "Y" connection. A mercury manometer attached to a 14 cm wide cuff was used.

Readings were always made in the morning in a quiet airconditioned room Recumbent pressures were measured after 15 minutes of rest and standing pressures were recorded 2 minutes after assumption of upright posture.

Recumbent and standing pulse rates were measured accordingly Phase V (disappearance) of Korotkoff sounds was taken as the diastolic end point. With few exceptions the average of 3 readings was calculated.

Clinic blood pressure was recorded at the beginning of the study after 4 weeks of placebo treatment and after 2 and 4 weeks of oral propranolol therapy

Home blood pressures

All patients were carefully instructed how to measure blood pressure and their accuracy was checked by the nurses using a "Y" connection. They were then provided with specially designed home blood pressure sphygomanometers (Propper) and instructed to record home blood pressure mornings and evenings in the recumbent and standing positions. Recumbent pulse rate was also recorded. Daily measurements of home blood pressure and pulse rate were continued throughout the study Special sheets were used for recording of these parameters and side-effects or comments. Home blood pressures were consistently lower than corresponding recordings in the clinic. Patient No 5 was an exception in this respect as his recordings at home were higher than in the clinic. Clinic blood pressures were significantly correlated to home blood pressure both after 4 weeks of placebo (Fig. 1) and after 4 weeks of propranolol (Fig. 2) independently of whether morning or evening home blood pressures were used.

Hospital investigations

After 4 weeks of placebo treatment all patients were hospitalized during 4 days in a metabolic research ward – the Clinical Research Unit for baseline laboratory studies. Placebo treatment was continued during the stay in hospital. Studies of 12-hour urinary excretion of catecholamines were made twice with collections starting at 8 p m A 24-hour endogenous creatinine clearance was made and, ending on the morning of the fourth day studies of 24-hour urinary excretion of aldosterone, sodium and potassium

A simple no-added-salt diet was introduced and the patients were instructed repeatedly by a dietician regarding the continuation of a similar diet following discharge.

An extensive number of blood (serum) and urine tests were made repeatedly during the study Ekg and chest x-ray were performed initially and following the placebo and propanolol periods.

additional dose of propranolol, 0.05 mg/kg, bringing the total dosage to 0 10 mg/kg. Car diac output was again determined after 10 min. Finally a further doubling of the dosage was made by giving a third injection of propranolol. To compensate for wear-off effects a 10% addi tion of dosage was made at this time bringing the cumulated dose to 0.22 mg/kg. Again hemodynamic measurements were repeated after 10 min.

A second 10-min period of 45° head-up tilt was then started at the end of which cardiac output measurements were repeated. Finally with the patients tilted back to horizontal, isoproterenol, 3 μg/min, was again infused intravenously during 3 min with recording of heart rate.

Hemodynamic study II

The second hemodynamic study was made at the same time of day and under similar conditions as the first study but this time following 4 weeks of oral propranolol therapy Cardiac output during rest and after 10 min of tilt was determined as before. Furthermore, the baroreceptor reflex was re-assessed. Finally the infusion of isoproterenol, 3 μg/min, over 3 min was repeated.

Nitroglycerine test

In order to evaluate the possibilities of estimating the degree of beta-adrenergic blockade by a non-invasive test nitroglycerine was given sublingually and the patients assumed upright posture while their pulse rate was recorded. This was done 4 times during the study

The nitroglycerine test was originally proposed by Fitzgerald as a simple means of estimating the degree of beta-adrenergic bloc kade [17] and he has also shown that the nitroglycerine test is more specific than e.g. Valsalva s manoeuvre, hyperventilation, sustained hand grip assumption of upright posture, mild exercise and maximal exercise because the effect of atropinization is less pronounced [18]

The nitroglycerine test as employed in the present study differed from Fitzgerald s original description in that the patients were told to stand up after the administration of nitroglycerine. This was made to ensure optimal stimulation of heart rate

After recording heart rate during rest the patients were given a nitroglycerine tablet, 0 4 mg, sublingually and at the same time they assumed upright posture. Pulse rate was then recorded continuously during 6 min and the highest pulse rate during any 30-second period was compared to the initial heart rate.

The test was performed in the same manner after four weeks of placebo treatment, after intravenous injection of propranolol, 0.22 mg/kg, (approximately 25 min after the injection was given) after two weeks of oral propranolol therapy at 160 mg daily (approximately 1 hour after the last dose) and finally after four weeks of oral propranolol therapy at 160 or 320 mg daily

The change of heart rate due to this manoeuvre was then compared to the change of heart rate due to isoproterenol infusion. It was also correlated to plasma propranolol concentration.

Plasma propranolol concentration

Plasma propranolol concentrations were deter mined by fluorimetric technique [87] by Ayerst Laboratories, Montreal, Canada through cour tesy of Dr Henry L. LeMien Jr

Blood samples were drawn approximately 20 min after the completion of the intravenous administration of propranolol 0.22 mg/kg and again following two and four weeks of oral propranolol therapy approximately 1-2 hours after the last dose.

The plasma was separated by centrifugation and chilled to 4° C before being sent in an isolated tube for analysis.

Plasma propranolol concentration was related to the change of blood pressure and other hemodynamic parameters as well as to changes

of plasma renin activity and to changes of heart rate due to sublingual nitroglycerine and intravenous isoproterenol. In view of recent observations [60] the logarithm of plasma propranolol concentration was also used for correlations.

Plasma renin studies
Analysis of plasma renin activity (PRA) was made after 4 weeks of placebo treatment in the recumbent position and again after 10 min of 45° head-up tilt. Furthermore, PRA was analysed after acute intravenous administration of propranolol 0.22 mg/kg and repeated tilt for 10 min. Finally PRA was determined following 4 weeks of oral propranolol therapy (160–320 mg daily) again after 10 min of tilt. Studies were not made in patient No 6.

For each sample 10 ml of arterial blood was collected in a prechilled tube containing approximately 10 mg of EDTA Na. The tube was immediately placed in ice and centrifuged within 20 min in a refrigerated centrifuge to recover the plasma. The plasma samples were then frozen and stored until all samples were collected.

For analysis a radioimmunoassay method was used [34, 63] by which PRA is determined by measurment of the generated angiotensin I* Duplicate analyses were made of all specimens and the results were compared to a standard curve obtained from standard solutions of angiotensin I Nonspecific activity was determined in specimen No 1 and subtracted from the results of analysis of samples No 1-3 (which were collected on the same day) Nonspecific activity was also determined in specimen No 4 and subtracted

The error of duplicate analyses varied between 4.2 and 7.9 % ($\sqrt{\frac{\Sigma d^2}{2n}}$ expressed in percent of the mean. d = the difference between duplicate analyses)

* Schwartz/Mann, Orangeburg, N Y

The change of stimulated (tilted) PRA after 4 weeks of oral propranolol therapy has been correlated to changes of blood pressure and other hemodynamic parameters.

Side effects
At each clinic visit patients were asked to report any adverse effects or complaints. They were then asked 17 questions from a standardized questionnaire relating to cardiopulmonary gastrointestinal, neurological and miscellaneous symptoms. A brief physical examination was also made at each visit directed at ruling out cardiac insufficiency and bronchial obstruction. Weight was recorded at each visit.

Statistical methods
Statistical methods included Student s t-test for group differences and paired data analysis, Wilcoxon s test and standard methods for calculation of correlation and regression coefficients (Documenta Geigy 1962) Most analyses were made using the computer program Midas (Constat) of the Statistical Research Laboratory of the University of Michigan. Probabilities less than 0 05 were considered significant.

Critical aspects of the material and methods

A. Effect of previous treatment
Eleven of the 15 patients were being treated with antihypertensive drugs before entering the study This therapy usually consisted of hydrochlorothiazide or alpha-methyldopa. In spite of the 4-week placebo period it is conceivable that some influence on blood pressure may have remained at the start of propranolol treatment although the effects should have been minimal and would tend to reduce the antihypertensive action of propranolol.

The effects on plasma renin activity are more interesting since both thiazides and alpha-methyldopa affect PRA. However it has been reported that the increase of PRA during chronic thiazide therapy is temporary and that PRA falls towards the initial level during treatment [3] Furthermore, it has been shown that elevated PRA falls to a normal level already during the first week after discontinuance of thiazide therapy [44] The effect of alpha-methyldopa on PRA is also relatively brief as illustrated by the finding that PRA had increased to the initial level 2 weeks after withdrawal of therapy [41] It can be concluded therefore that the effects of previous therapy were insignificant.

B Use of placebo treatment

It can be argued from an ethical point of view that, with our present knowledge of hypertension, placebo should not be given. In the present study attempts were made to minimize the effects of withdrawal of active therapy by selecting patients with relatively mild hypertension and by limiting the placebo period to 4 weeks. Furthermore, an extra precaution was taken by providing all patients with equipment for measurement of home blood pressure and instructing them to call if diastolic blood pressures over 120 mm Hg were recorded repeatedly

C. Effect of age

The age distribution in the present study (26–66 years, mean 45 median 46) was fairly wide However age was evenly distributed. It has been shown that older subjects have lower resting cardiac output and higher total peripheral resistence [37] It is also known that age reduces the hemodynamic effects of propranolol [7] For these reasons, age has been correlated to changes of blood pressure and other hemodynamic alterations as well as to changes of plasma renin activity In no instance have significant correlations been observed between age and alterations of hemodynamic parameters or PRA This indicates that age did not affect the results of the present study Finally as comparisons are made on a within-patient basis and few attempts are made to compare groups the importance of age is further reduced

D Effects of blood loss

As blood was not reinfused during the hemodynamic studies and as a number of blood tests and sampling of blood for PRA was was made during the study it is conceivable that a reduction of hemoglobin concentration or hematocrit might occur which could affect blood viscosity and thereby limit the possibilities of comparing e.g. TPR before and after oral propranolol therapy For this reason hemoglobin concentration and hematocrit before the first and after the second hemodynamic study were compared statistically (paired t test) No significant changes were seen of either parameter indicating that significant changes of blood viscosity had not occured.

RESULTS

Effect on blood pressure

Significant reductions of blood pressure were seen both with regard to measurements made in the clinic (Table II) and at home (Table III). A significant reduction of home blood pressure was seen already during the first week of propranolol therapy but there was an insignificant further fall of blood pressure also during the following weeks. Individual changes of clinic blood pressure are given in Fig. 3 and 4.

Pulse rate was also significantly reduced both in the recumbent and standing position. (Table II)

The changes of systolic blood pressure (recumbent and standing) as measured in the clinic after 4 weeks of oral therapy were significantly correlated to plasma propranolol concentration ($r = 0.782$, $p < 0.005$ and $r = 0.541$ $p < 0.05$). The changes of diastolic blood pressure recumbent or standing were not significantly correlated to plasma propranolol concentration, ($r = 0.358$, n.s. and $r = 0.283$ n.s.) Furthermore, the changes of both systolic and diastolic blood pressure, recumbent and standing, between the second and fourth week of propranolol therapy were significantly correlated to plasma propranolol concentration after 4 weeks of treatment (correlation coefficients between 0.525 and 0.659 $p < 0.05$ to $p < 0.01$)

Blood pressure changes in the 8 patients treated with 160 mg daily for 4 weeks were similar to those of the group in which dosage was increased to 320 mg. However a tendency towards continuing reduction of systolic blood pressure was observed in the latter group (Fig. 5)

Table II. **Change of clinic blood pressure and heart rate.**

	Recumbent			Standing		
	Heart rate	BP syst.	BP diast.	Heart rate	BP syst.	BP diast.
Initial*	77.9	150.8	102.3	88.4	148.4	106.8
Placebo**	82.9	164.3	107.7	92.5	158.1	111.2
Difference	5.0	13.5	5.4	4.1	9.7	4.4
Significance p <	n.s.	0.05	n.s.	n.s.	n.s.	n.s.
After 2 weeks of oral Propranolol	67.8	136.7	91	74.3	137.3	97.7
Difference***	15.1	27.6	16.6	18.2	20.8	13.5
Significance p <	0.0001	0.0001	0.0005	0.0001	0.001	0.005
After 4 weeks of oral Propranolol	64.6	135.7	87.8	68.8	135.4	95.9
Difference***	18.3	28.6	19.9	23.7	22.7	15.2
Significance p <	0.0001	0.0001	0.0001	0.0001	0.0001	0.0005

At admission to study **Following 4 weeks of placebo treatment
***In comparison to Placebo. Statistical comparison with paired data t-test

Table III **Change of recumbent home blood pressure and heart rate**

	Morning			*Evening*		
	Heart rate	BP syst.	BP diast.	Heart rate	BP syst.	BP diast
Week 4 Placebo	74.0	147.5	101.6	79.6	148.1	98.8
Week 1 Propranolol	69.8	134.0	88.7	72.5	132.3	89.0
Difference	4.2	13.5	12.9	7.1	15.8	9.8
Significance p <	0.01	0.001	0.0001	0.001	0.0005	0.005
Week 2 Propranolol	68.7	132.9	86.6	72.4	132.4	86.8
Difference	5.3	14.6	15.0	7.2	15.7	12.0
Significance p <	0.005	0.005	0.0005	0.005	0.0001	0.0005
Week 3 Propranolol	69.7	131.3	85.1	72.7	127.3	84.9
Difference*	4.3	16.2	16.5	6.9	20.8	13.9
Significance p <	0.01	0.001	0.0001	0.005	0.0001	0.0005
Week 4 Propranolol	67.0	133.1	86.7	70.5	129.9	83.9
Difference	7.0	14.4	14.9	9.1	18.2	14.9
Significance p <	0.005	0.001	0.0001	0.005	0.0001	0.0001

*In comparison to week 4 of placebo treatment. The average of seven recordings per week are given. Statistical comparison with paired data t-test.

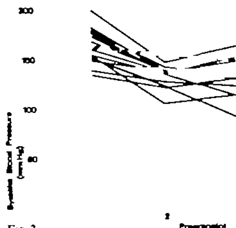


Fig 3

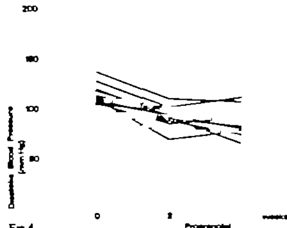


Fig 4

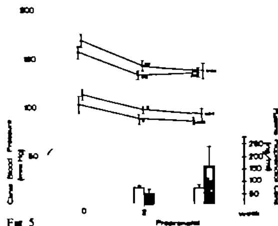


Fig 5

Fig 3
Individual changes of recumbent systolic blood pressure measured in the clinic. Initial recordings were after 4 weeks of placebo treatment. Propranolol dosage was 160 mg daily during weeks 1 2 and 160 or 320 mg daily (av 250) during weeks 3–4

Fig 4
Individual changes of recumbent diastolic blood pressure measured in the clinic. Therapy as in Fig 3

Fig 5
Clinic blood pressure and plasma propranolol concentration in patients receiving propranolol 160 mg daily for 4 weeks (circles and unfilled bars) and in patients receiving 160 mg daily for 2 weeks and 320 mg daily for 2 weeks (triangles and striped bars). Mean values ± S E M. Statistical comparisons are within the groups (paired t-test). Probabilities as in Fig 6

Hemodynamic effects

Resting data during placebo

During placebo treatment the following hemodynamic parameters (± S.E.M.) were recorded (Table IV)

1) Qi 2.76 ± 0.15 l/min m²
2) MAP 108.3 ± 2.5 mm Hg.
3) TPRI 41.3 ± 2.7 u/m²
4) HR 76.0 ± 2.3 beats/min.
5) SVI 37.3 ± 2.6 ml/m²

Effects of acute intravenous propranolol

Intravenous administration of propranolol, 0.05 mg/kg, resulted in (Fig. 6 and 7)

1) Qi fell from 2.76 to 2.29 l/min m² ($p<0.01$)
2) MAP did not change significantly at 107.2 mm Hg.
3) TPRI increased from 41.3 to 48.5 u/m² ($p<0.05$)
4) HR fell from 76.0 to 71.8 beats/min ($p<0.005$)
5) SVi did not change significantly at 32.4 ml/m²

After doubling the dose of propranolol to a total cumulated dosage of 0.10 mg/kg the

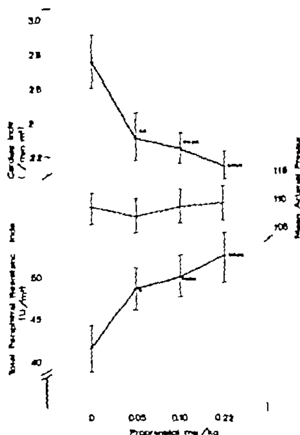


Fig. 6
Effects of increasing doses of propranolol I.V. on cardiac index, mean arterial pressure (dashed lines) and total peripheral resistance index. The cumulated doses indicated on the x-axis were given 10 min apart. Mean values ± S.E.M. are given. $^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.005$ $^{****}p<0.001$ (paired t-test) Comparisons to initial values

Table IV Hemodynamic changes at rest.

	Placebo	Propranolol 0.22 mg/kg I.V.	Difference	Significance p <	Propranolol orally*	Difference**	Significance p <
Cardiac index l/min m²	2.76	2.18	0.58	0.001	2.35	0.41	0.01
Mean arterial pressure mm Hg	108.3	109.9	1.7	n.s.	102.1	6.2	0.05
Total peripheral resistance index u/m²	41.3	52.3	11.0	0.005	43.9	2.6	n.s.
Heart rate beats/min	76.0	67.1	8.9	0.0001	59.6	16.4	0.0001
Stroke volume index ml/m²	37.3	33.0	4.3	n.s.	40.2	2.9	n.s.

*Four weeks of oral treatment 160–320 mg daily. **Compared to measurement after placebo

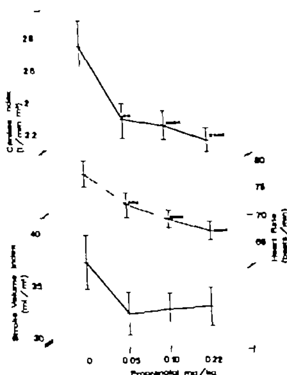


Fig 7
Effects of increasing doses of propranolol I V on cardiac index, heart rate (dashed lines) and stroke volume index. Dosage and statistics as in Fig 6

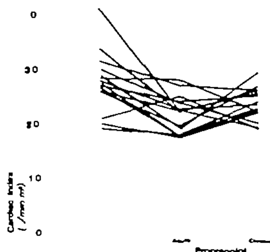


Fig 8
Individual changes of resting cardiac index. Initial measurements are after 4 weeks of placebo treatment. Acute propranolol indicates measurement after I V administration of 0 22 mg/kg. Chronic propranolol indicates 4 weeks of oral treatment 160–320 mg daily

hemodynamic results were (comparisons to the corresponding data during placebo)

1) Qi fell to 2.25 l/min m² ($p<0.001$)
2) MAP did not change significantly at 109 1 mm Hg.
3) TPRi increased to 49.9 u/m² ($p<0\ 001$)
4) HR fell to 69 4 beats/min ($p<0\ 0001$)
5) SVi did not change significantly at 32.7 ml/m² (Fig. 6 and 7)

By further increasing the dosage of propranolol to a cumulated dosage of 0.22 mg/kg the hemodynamic results were (comparisons to the corresponding data during placebo)

1) Qi fell to 2.18 l/min m² ($p<0.001$)
2) MAP did not change significantly at 109.9 mm Hg.
3) TPRi increased to 52.3 u/m² ($p<0\ 001$)
4) HR fell to 67 1 beats/min ($p<0\ 001$)
5) SVi did not change significantly at 33 0 ml/m² (Fig. 6 and 7)

Effects of prolonged oral propranolol

Four weeks of oral propranolol therapy resulted in (Table IV and Fig. 9)

1) A remaining significant reduction of Qi (from 2.76 to 2.35 l/min m² $p<0\ 01$)
2) A significant reduction of MAP from 108.3 to 102.1 mm Hg ($p<0.05$)
3) A re-adjustment of TPRi to a level not significantly higher than during placebo treatment (43 9 u/m²)
4) A significant reduction of HR to 59 6 beats/mm ($p<0\ 0001$) This was also significantly lower than HR after acute I V propranolol
5) A non-significant increase of SVi to 40.2 ml/m²

Individual changes of cardiac index are illustrated in Fig. 10.

Analysis of the hemodynamic changes in individual patients revealed that while 12 patients showed a decrease of MAP following prolonged oral propranolol therapy 3 patients

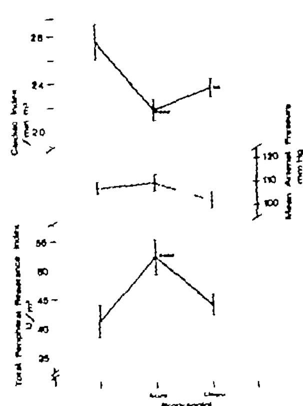


Fig 9
Effects of acute I V (0.22 mg/kg) and 4 weeks of oral propranolol (160–320 mg daily) on cardiac index, mean arterial pressure (dashed lines) and total peripheral resistance index. Statistics as in Fig. 6

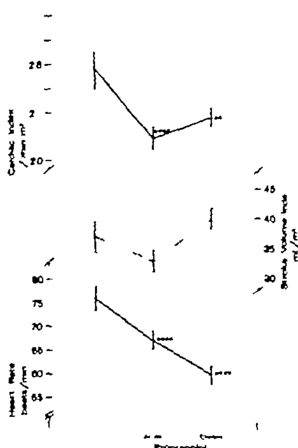


Fig. 10
Effects of acute I V (0.22 mg/kg) and 4 weeks of oral propranolol (160–320 mg daily) on cardiac index, stroke volume index (dashed lines) and heart rate
Statistics as in Fig. 6

Table V Hemodynamic changes during tilt.

	Placebo (n = 15)				*Propanolol I V* (n = 12)				*Propanolol orally*** (n = 12)			
	Rest	Tilt	Difference	Significance p<	Rest	Tilt	Difference	Significance p<	Rest	Tilt	Difference	Significance p<
Cardiac index l/min m²	2.76	2.19	0.57	0.005	2.19	1.60	0.59	0.0005	2.44	2.04	0.40	0.005
Mean arterial pressure mm Hg	108.3	119.6	11.3	0.0005	106.4	98.3	8.1	n.s	103.4	110.3	6.9	0.005
Total peripheral resistance index u/m²	41.3	56.5	15.2	0.0001	50.4	63.8	13.4	0.005	42.6	54.6	10.0	0.0005
Heart rate beats/min	76.0	84.9	8.9	0.005	69.5	69.8	0.3	n.s.	57.7	60.6	2.9	0.01
Stroke volume index ml/m²	37.3	26.4	19.9	0.0001	32.0	22.9	9.1	0.0001	42.7	34.4	8.3	0.0005

*0.22 mg/kg I V **oral treatment for 4 weeks, 160–320 mg daily

(No 10, 11 and 12) showed no decrease or an increase. By comparing the two groups it was shown that there was no significant difference of Qi and TPRi during the placebo period. Neither was Qi significantly different after oral propranolol treatment. However the difference of MAP was reflected by a significant difference of TPRi after oral propranolol therapy MAP was also significantly different between the two groups already during acute beta-adrenergic blockade (105.9 vs 122.2 mm Hg, $p<0.05$) (Fig. 11)

None of the hemodynamic changes (except for the previously mentioned reductions of clinic blood pressure) were significantly correlated to plasma propranolol concentration after 4 weeks of oral therapy

Effects of tilt

A After placebo

There were no distressing symptoms during the initial 10 min tilt. As expected, there were reductions of Qi and SVi while HR and TPRi rose significantly resulting in a rise of MAP (Table V)

B After I V propranolol

Tilt after intravenous administration of propranolol caused disturbing postural symptoms in several patients and one patient actually fainted just after the hemodynamic measurements were made. In comparison to the hemodynamic parameters in the recumbent position after I V propranolol significant reductions of SVi and Qi occurred while TPRi increased. The net effect on MAP was a non-significant reduction (Table V)

C After oral propranolol

There were no complaints of discomfort during tilt after 4 weeks of propranolol therapy and no patient appeared to be close to fainting. Again significant reductions of SVi and Qi

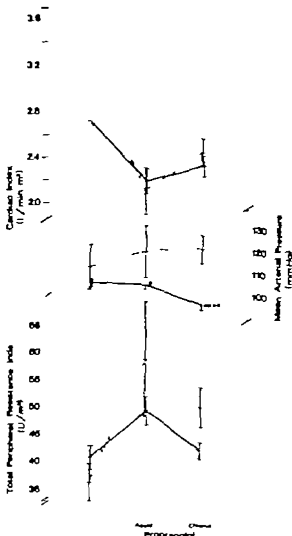


Fig 11
Hemodynamic differences between 12 patients with reduced intra-arterial pressure and 3 with unchanged or increased MAP (dashed lines) Mean values ± S E M are given Probabilities as in Fig 6 (Student's t-test) Comparisons between groups

and a significant increase of TPRi were seen As in the placebo situation, there was an increase of MAP and a small but significant increase of HR (Table V)

Effect on baroreceptor sensitivity

Only in 7 patients were statistically significant correlations between intra-arterial systolic

blood pressure and R-R interval (BP/R-R slope) obtained following I V angiotensin both after placebo and after 4 weeks of oral propranolol therapy The average initial regression coefficient was 4 84 After treat ment the average regression coefficient was 7 64 The difference, 2.80 did not quite achieve statistical significance ($p = 0.115$)(paired t-test)

The change of the BP/R-R slope was not significantly correlated to plasma propranolol concentration.

Effect of Isoproterenol Infusions

In the untreated state, recumbent resting heart rate increased from 73 1 to 85 2 beats/min ($p<0\ 0005$) in response to intravenous infusion of isoproterenol 1 μg/min during 3 min. By increasing the rate of infusion to 2 μg/min for 3 min heart rate increased to 100 7 beats/min ($p<0\ 0005$) Finally following 3 min of isoproterenol infused at a rate of 3 μg/min heart rate accelerated to 112.8 beats/min bringing the total increase to 39 8 beats/min ($p<0\ 0005$)

Intravenous infusion of isoproterenol at the rate of 3 μg/min for 3 min was repeated after intravenous administration of propranolol, 0 22 mg/kg. In this situation average heart rate only increased by 0.9 beats/min (n.s.) indicating a marked degree of beta-adrenergic blockade

Finally after 4 weeks of oral propranolol therapy isoproterenol was again infused at a rate of 3 μg/min over 3 min The average increase of heart rate was only 5 3 beats/min ($p<0\ 05$) again indicating marked beta-adrenergic blockade

Effect on plasma renin activity

All patients were within the normal or low normal range in regard to resting plasma renin activity (PRA) after placebo. In response to 10 min of 45° head -up tilt average PRA rose from 151 7 to 248 7 ng/100 ml h (n.s.) Renew

Table VI **Plasma renin activity (PRA) and sodium excretion.**

Patient	PRA rest placebo	Urinary Sodium mEq/ 24 h	PRA tilt placebo	PRA tilt prop. I.V **	PRA tilt prop orally***
1	40.3	160	59 4	27.6	14.0
2	228 2	67	434 0	318 0	89.2
3	54.4	105	149.0	68.9	12.4
4	443 4	55	709.3	722.9	20.4
5	48.8	46	52.7	N.A.	10.7
7	461.3	171	875.6	739 6	93 4
8	45 4	158	68 5	32.6	38.1
9	113.3	94	135.9	60.9	16.5
10	191 9	N.A.	146.4	93.0	6.9
11	125 4	N.A.	151 4	76 2	39 7
12	51 7	58	82.4	49 7	7.9
13	76 5	101	148.4	142.5	90.1
14	704 7	68	406 5	304 7	104 4
15	37 9	93	6..2	24.9	1 7
Average	151 7	98.0	248 7	190 0	39.0
S.E.M	± 38 2	± 12.6	± 69.8	± 66.5	± 10.1

Significance ________ n s ________

________ n.s ________

________ p <0 005 ________

________ p <0 005 ________

*ng/100 ml h
**Propranolol 0.22 mg/kg I V ***Propranolol orally for four weeks, 160-320 mg daily N.A = Not available.

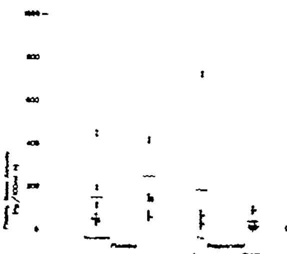


Fig 12
Plasma renin activity during placebo (resting recumbent and after 10 min 45° tilt) after propranolol 0 22 mg/kg I V (and repeated tilt) and finally following 4 weeks of oral propranolol therapy (and repeated tilt) Horizontal lines indicate mean values

ed tilt after acute intravenous administration of propranolol 0.22 mg/kg caused a smaller increase of PRA to 190.0 ng/100 ml h (n.s.)

Finally after 4 weeks of oral propranolol therapy average PRA follwing 10 min of 45° tilt was only 39.0 ng/100 ml h. This is significantly lower than both the initial recumbent level ($p<0.005$) and the level after initial tilt ($p<0.005$)

Thus, the average stimulated (tilted) PRA following 4 weeks of oral propranolol was only 15% of the corresponding PRA during placebo Individual PRA after prolonged propranolol therapy were all in the low or very low range (Fig. 12 and Table VI)

Change of PRA in relation to hemodynamic alterations

The change of tilted PRA from placebo to the level after oral propranolol treatment (average 209.3 ng/100 ml h) was not significantly correlated to either the reduction of recumbent systolic blood pressure as measured in the clinic ($r = 0.230$ n.s.) or to the change of diastolic recumbent blood pressure ($r = 0.449$ n.s.)

Nor was the change of tilted PRA significantly correlated to the changes of intra-arterial MAP Qi or TPRi either in the recumbent or tilted position, (correlation coefficients 0.077–0.104 n.s.)

Table VII **Correlations between decrease of home blood pressure (systolic and diastolic blood pressure measured in the recumbent and standing position mornings and evenings) and various initial parameters.**

	Correlation coefficients		Probabilities		Remarks
Age	0.068	0.336	> 0.25		
Weight	0.058	0.442	> 0.25	< 0.20	
Heart Rate	0.046	0.663	> 0.25	< 0.02	1 of 8 correlations significant
Cardiac output	0.013	0.419	> 0.25	< 0.15	
Cardiac index	0.018	0.340	> 0.25		
Total peripheral resistance	0.154	0.700	> 0.25	< 0.02	2 of 8 correlations significant
Response to beta-adrenergic stimulation	0.314	0.589	> 0.25	< 0.05	1 of 8 correlations significant
Norepinephrine** excretion in urine	0.021	0.370	> 0.25	< 0.20	
Epinephrine** excretion in urine	0.007	0.395	> 0.25	< 0.20	
Total catecholamine** excretion in urine	0.013	0.419	> 0.25	< 0.15	
Aldosterone*** excretion in urine	0.211	0.591	> 0.25	< 0.05	1 of 8 correlations significant
Creatinine clearance	0.014	0.408	> 0.25	< 0.20	

*I.V. infusion of isoproterenol. **Two 12 h urine collections ***One 24 h urine collection.

Furthermore, the change of tilted PRA, expressed in percent, was not significantly correlated to the percentage changes of MAP QI or TPRi either recumbent or tilted, (correlation coefficients 0 010–0.387 n.s.)

The individual responses were very similar particularly in regard to the effect of 4 weeks of oral propranolol. For example, the 3 patients showing unchanged or elevated intra-arterial pressure and significantly higher TPRi after oral propranolol did not show a different pattern in regard to PRA as their reductions of tilted PRA were 81 90 and 96 % respectively as compared to the average of 85 %

Finally the changes of PRA were not significantly correlated to plasma propranolol concentrations nor was the final level of PRA correlated to plasma propranolol concentration after 4 weeks of oral propranolol therapy

Observations on predictability

As one of the aims of the present study was to try to identify factors that might help to predict the antihypertensive effect of propranolol a number of initial parameters were correlated to the change of blood pressure during oral propranolol therapy Both clinic and home blood pressures, (systolic and diastolic, recumbent and standing, mornings and evenings) were used for analysis. Thus, the change of blood pressure

Table VIII **Correlations between decrease of clinic blood pressure (systolic and diastolic blood pressure measured in the recumbent and standing position) and various initial parameters.**

	Correlation coefficients		Probabilities		Remarks
Age	0 074	0.489	> 0.25	< 0.15	
Weight	0 062	0 491	> 0.25	< 0 15	
Heart rate	0 001	0 440	> 0.25		
Cardiac output	0 006	0.267	> 0.25		
Cardiac index	0.047	0.246	> 0 25		
Total peripheral resistance	0 058	0 229	> 0.25		
Response to beta-adrenergic stimulation*	0.102	0.341	> 0 25		
Norepinephrine** excretion in urine	0 015	0 389	> 0.25	< 0 20	
Epinephrine* excretion in urine	0 093	0 457	> 0 25	< 0 15	
Total catecholamine** excretion in urine	0 047	0 453	> 0 25	< 0 15	
Aldosterone*** excretion in urine	0 024	0 187	> 0 25		
Creatinine clearance	0 018	0 321	> 0 25		

LV Infusion of isoproterenol **Two 12 h urine collections ***One 24 h urine collection.

during propranolol was expressed in 12 different ways. As illustrated in Table VII and VIII significant correlations to 11 initial parameters occurred only at random (5 statistically significant correlations out of 132 calculations) indicating that none of the selected parameters was useful for prediction of response.

Usefulness of the nitroglycerine test for estimation of beta-adrenergic blockade

After 4 weeks of placebo sublingual administration of nitroglycerine 0 4 mg and change to upright posture caused an increase of pulse rate from 80.3 to 115 4 beats/min, (difference 35 1 $p<0\ 0005$) (Fig. 13)

As previously mentioned, intravenous infusion of isoproterenol 1 μg/min over 3 min increased heart rate by 12.1 beats/min. The response to nitroglycerine was not significantly correlated to the effect of isoproterenol at the infusion rate of 1 μg/mm (r = 0.351, n.s.) However the increase of heart rate during infusion of isoproterenol at the rate of 2 μg/min (27 6 beats/min) was significantly correlated to the nitroglycerine effect (r = 0.510, $p<0.05$)

Finally the increase of heart rate during infusion of isoproterenol at the rate of 3 μg/min (39 8 beats/min) was also significantly correlated to the effect of nitroglycerine (r = 0 633 $p<0\ 005$)

The nitroglycerine test was not carried out after acute I V administration of propranolol due to severe postural effects in 3 of the first 4 patients.

Following two weeks of oral propranolol therapy at 160 mg daily the average increase of pulse rate due to sublingual nitroglycerine and upright posture was 21.5 beats/min ($p<0\ 005$) This was inversly correlated to plasma propranolol concentration (r = - 0.518, $p<0\ 05$) The increase of pulse rate was not significantly correlated to the logarithm of plasma propranolol concentration (r = - 0.398, n.s.)

Following 4 weeks of oral propranolol therapy (av 235 mg/d) the average increase of pulse rate after sublingual nitroglycerine and upright posture was 17.3 beats/min ($p<0.005$)

The change of heart rate in response to I V isoproterenol, 3 μg/min, was 5.3 beats/min ($p<0.05$) Again the effects of nitroglycerine and isoproterenol were significantly correlated (r = 0.611 $p<0.01$) However as opposed to the results during placebo there now was a marked difference between the two manoeuvres in regard to the quantitative response (Fig. 13)

The average plasma propranolol concentration after 4 weeks of oral treatment was 123 1 ± 46 1 ng/ml but this was not significantly correlated to the nitroglycerine induced increase of pulse rate (r = - 0.184 n.s.) Nor was the logarithm of plasma propranolol concentration significantly correlated to the effect of nitroglycerine (r = - 0 010, n.s.)

Furthermore, plasma propranolol concentration, or its logarithm, was not significantly correlated to the effect of isoproterenol infusion (r = - 0.384 n.s. and r = - 0.386, n.s.)

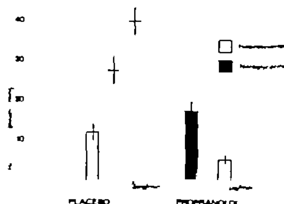


Fig. 13
Average increase of heart rate ± S.E.M in response to sublingual nitroglycerine 0 4 mg and assumption of upright posture and intravenous infusion of isoproterenol at the rate of 1, 2 and 3 μg/min Placebo indicates that the tests were performed after 4 weeks of placebo treatment. Propranolol indicates that the tests were performed after 4 weeks of oral propranolol therapy

The effect of 10 min of 45° head-up tilt was not correlated to the effect of nitroglycerine either during placebo (r = 0.308, n.s.) or after 4 weeks of oral propranolol treatment (r = 0 418, n.s.) Nor was the effect of tilt correlated to the effect of isoproterenol either before or during propranolol therapy (r = 0.272, n.s. and r = 0.238, n.s.)

Plasma propranolol concentration

Average plasma propranolol concentration after acute I V administration of propranolol 0.22 mg/kg was 100.5 ± 6.0 ng/ml and as indicated by the S.E.M individual variations were small. Following 2 weeks of oral propranolol at 160 mg daily the average plasma concentration was 68.3 ± 11 5 ng/ml and finally after 4 weeks of oral therapy average plasma propranolol concentration was 123 1 ± 46.1 ng/ml It has already been indicated that the reduction of systolic blood pressure in the clinic was significantly correlated to plasma propranolol concentration after 4 weeks of oral therapy However the degree of beta adrenergic blockade was not directly correlated to plasma propranolol levels, as indicated by the response to isoproterenol infusions.

Patients receiving propranolol 160 mg daily during 4 weeks had an average plasma propranolol concentration of 68.0 ± 7 1 ng/ml after two weeks and 69 1 ± 17 0 ng/ml after 4 weeks. As expected, plasma propranolol concentration rose in the patients given 320 mg daily during the last 2 weeks. Average plasma propranolol concentration in this group after 4 weeks was 162.7±82.6 ng/ml (Fig. 5) Due to the wide individual variations this change did not achieve statistical significance.

In summary plasma propranolol concentration at the end of 4 weeks of oral propranolol therapy was significantly correlated to the over all reduction of systolic recumbent and standing blood pressure as measured in the clinic Except for this, plasma propranolol concentration was not significantly correlated to changes of hemodynamic parameters or plasma renin activity Nor was the degree of beta-adrenergic blockade, as estimated by heart rate response to infusion of isoproterenol or sublingual nitroglycerine, correlated to plasma propranolol concentrations.

SIDE EFFECTS

Only one patient had side effects from the treatment with propranolol. Patient No 9 complained of insomnia, being able to sleep only a few hours every night. He also experienced tiredness during the day possibly secondary to the lack of sleep For this reason he was offered a change of therapy but volunteered to continue propranolol treatment. Subjectively some improvement occurred during continued treatment. This patient was clearly normotensive at the end of the study having a recumbent blood pressure of 115/80 mm Hg in the clinic.

With this exception, no side effects were reported or observed. No consistent changes of weight were observed nor were there any signs of development of latent or manifest cardiac decompensation. Heart size or pulmonary venous pattern did not change radiographically EKG changes were confined to the expected reduction of heart rate.

Repeated studies of hematological tests, renal and liver function tests as well as a number of other blood and urinary tests did not reveal any consistent changes.

As expected, postural hypotension or hypotensive symptoms during physical activity did not occur

DISCUSSION

Antihypertensive effect

The antihypertensive effect observed in the present study is in agreement with previously published reports in this field,[25, 57, 81] indicating that beta-adrenergic blockade with propranolol in hypertensive patients is effective in reducing arterial pressure with few or no side effects and no postural hypotension. Contrary to some reports,[25, 51] the antihypertensive action appeared to be of rapid onset as judged from the daily measurements of home blood pressure. This may be a result of the relatively high dosage (40 mg 4 times daily) that was used already from the first day of active treatment.

It is entirely possible that the dosages and the duration of treatment employed in the present study have not produced the optimal antihypertensive effect obtainable in every individual patient. However, based on the results in this series of patients with mild to moderately severe essential hypertension, it can be concluded that propranolol has a useful antihypertensive action.

Hemodynamic effects

The antihypertensive effect of propranolol may seem surprising in view of the fact that the drug fails to reduce arterial pressure after acute intravenous administration,[78] a finding that is confirmed in this study. It has been shown that when given acutely, propranolol reduces cardiac output mainly through a reduction of heart rate[78] and this is also demonstrated in the present series. In the acute situation, total peripheral resistance is increased in response to the reduction of cardiac output and consequently arterial pressure remains unchanged. It has been postulated that the antihypertensive effect seen during chronic propranolol therapy must be attributed to prolonged reduction of cardiac output.[20] As shown in the present series, cardiac output remains at a lower level during oral propranolol therapy but total peripheral resistance is readjusted to the initial level thereby reducing blood pressure.

It has even been shown in a recent study that total peripheral resistance may fall below the initial level during long-term therapy.[66] The mechanism by which this re-adjustment of total peripheral resistance occurs is not fully understood. Initially it was postulated that a resetting of baroreceptor sensitivity took place.[80, 81] However, others rejected this hypothesis based on the erroneous assumption that total peripheral resistance remained elevated during chronic propranolol therapy.[20]

The demonstration that peripheral resistance is re-adjsuted to the initial level – or lower – makes it theoretically feasible that baroreceptors are reset. In fact, the data presented in the present study suggest that such a resetting towards a greater baroreceptor sensitivity takes place. It should be pointed out, though, that the studies on baroreceptor sensitivity as performed in the present study are concerned only with the effect on heart rate in response to a temporary increase of blood pressure. There is no reason to believe that a resetting of baroreceptor sensitivity would affect the regulation of heart rate only and

leave vasomotor control unaffected. The baroreceptor reflex was not assessed after acute beta-adrenergic blockade. However others have reported some increase of baroreceptor sensitivity after I.V propranolol but a total abolishment of the reflex after atropine [83]. This would indicate that the reduction of heart rate in response to an acute elevation of blood pressure is effected mainly by increased vagal stimulation and not by withdrawal of beta adrenergic stimulation. It therefore would appear to be justified to compare the baroreceptor sensitivity during placebo treatment to that during prolonged propranolol therapy. Furthermore, the findings in the present study do not demonstrate a statistically significant change of baroreceptor sensitivity probably due to the fact that only 7 pairs of observations were used.

If however baroreceptors were reset, the explanation for this is not immediatly evident as blood pressure is not reduced after acute beta-adrenergic blockade. It it possible that propranolol would reduce the cardiac component of transient blood pressure peaks occurring throughout the the day and thereby cause a reduction of average blood pressure during the day. This could result in a subsequent resetting of baroreceptor sensitivity. The importance of changes of flow characteristics during propranolol therapy and the prolongation of diastole due to the reduction of heart rate remains speculative in regard to its importance for baroreceptor adjustments.

It is also conceivable that central nervous effects of propranolol may be responsible for a modified efferent outflow from the vasomotor center e.g. in response to afferent baroreceptor impulses, resulting in a reduction of peripheral vascular resistance. Undoubtedly further research is needed before we understand the exact mechanism underlying the demonstrated re-adjustment of peripheral resistance.

As previously mentioned, the local anesthetic effect of propranolol obviously plays a minor role in the antihypertensive action as indicated by the lack of hypotensive effect of d propranolol [75]. Furthermore, if lowering of blood pressure were to result from anesthesia of vasoconstrictor fibres, it would be expected that acute intravenous administration of propranolol should not elevate TPR. In the present series all patients but three showed a reduction of intra-arterially measured mean pressure, due to a significant difference in total peripheral sistance. However the importance of this finding is doubtful as the reduction of clinic and home blood pressures were of the same magnitude in these 3 patients as in the remaining group. It is conceivable that the antihypertensive effect was masked in the hemodynamic situation, e.g. due to anxiety.

The effect of tilt

Tilt during placebo produced a reduction of cardiac output and stroke volumes of the same order that has been reported previously in normal subjects and patients with border line hypertension [84]. Similar changes were seen after intravenous and oral propranolol therapy. However the reflex increase of heart rate was abolished after acute beta-adrenergic blockade and reduced after prolonged oral propranolol therapy. The change of cardiac output due to tilt was of the same magnitude after intravenous propranolol as during placebo. However as this *change started* from an already reduced level of cardiac output after acute beta-adrenergic blockade, dizziness and other signs of insufficient cerebral blood flow occurred. As mentioned elsewhere heart rate response to tilt was not a useful indicator of the degree of beta-adrenergic blockade.

Effect on plasma renin activity

The mechanisms underlying variations of renin secretion are not fully known and several theories including the existence of intrarenal baroreceptors, volume receptors and chemoreceptors have been suggested. However after the demonstration of an inhibitory influence of several anti-adrenergic drugs [41, 44, 80] interest has been directed to the adrenergic mechanisms involved in renin secretion. In the present study acute beta-adrenergic blockade reduced the effect of tilt on plasma renin activity However the effect of 4 weeks of oral propranolol therapy was much more striking, resulting in reductions of PRA to low or very low levels in all patients. It would appear therefore, that renin release is mediated largely through beta-adrenergic receptors.

In the present series of essential hypertensives there was no correlation between reduction of PRA and reduction of blood pressure. Nor was the initial level of PRA related to the response to propranolol. This is in contrast to a recent report by Bühler et al. in which patients with high PRA were shown to have a greater reduction of diastolic blood pressure during propranolol therapy than patients with low PRA [6] However as Bühler's series included patients with renal and malignant hypertension, and as no patient in the present series had high PRA no direct comparisons can be made

Recently it has been shown that marked reductions of PRA takes place after only 3 days of oral propranolol therapy while little or no effect on blood pressure occurs [40] Others have stated that blood pressure response to propranolol can not be ascribed in the majority of patients to differences in its effect on PRA [88] This is in agreement with the results of the present study

With regard to the previously discussed mechanisms underlying the re-adjustment of peripheral vascular resistance it could be argued from a theoretical point of view that the reduction of PRA – and consequently of angiotensin – might reduce peripheral vasoconstriction and thereby lower TPR. However as PRA usually is not elevated in patients with benign essential hypertension – including the patients of the present series – the increased vascular resistance in this form of hypertension is most likely independent of this mechanism. This is further supported by the results of the present study in which no significant correlation was seen between reduction of PRA and reduction of total peripheral resistance.

The conclusion therefore must be that the reduction of PRA and the reduction of blood pressure (and peripheral resistance) in essential hypertension during propranolol therapy seem to be two separate phenomena that are not directly linked together If low PRA constitutes a protection against cardiovascular risks, e.g. myocardial infarction or stroke as indicated in a recent study [6] and if drug induced reduction of PRA will reduce such risks cannot be commented upon at the present time. Clearly this field deserves further research.

Observations on predictability

There is always a desire in medical therapy to make predictions regarding the response to therapy or even to select patients based on predictive judgement. In the present study correlations were made between the reduction of blood pressure and 11 initial parameters for the purpose of identifying factors that might help predict the response to propranolol. From a logical point of view some of the selected parameters could be expected to serve a useful purpose in this respect.

Obviously initial heart rate, cardiac output or cardiac index would seem to be factors worth studying. However hypertensive patients with high cardiac output have not responded more favourably to propranolol in previously published studies [2, 88] and the the

results of the present study confirm this. It would seem appropriate therefore to state that administration of propranolol to hypertensive patients should not be based on whether these patients have hyperkinetic or normokinetic circulation.

Another seemingly logical factor to test would be the response to beta-adrenergic stimulation prior to administration of beta adrenergic blockade. However the heart rate response to intravenous administration of isoproterenol was not significantly correlated to the reduction of blood pressure during propranolol therapy

The lack of correlation between urinary catecholamine excretion and response to propranolol may not be all that surprising in view of the fact that increased excretion of norepinephrine is not a characteristic finding in essential hypertension [16] On the other hand it has recently been shown that patients with essential hypertension have increased levels of plasma catecholamines [10, 14] and it should be stressed that simply studying the urinary excretion of these substances may be too crude to evaluate their importance in essential hypertension.

Finally the lack of correlation between blood pressure reduction and the other parameters (age, weight, total peripheral resistance, aldosterone excretion and creatinine clearance) only leads to the conclusion that none of the employed parameters served the desired purpose of predicting the antihypertensive response to propranolol.

Estimation of beta-adrenergic blockade

Logically the way to test beta-adrenergic blockade is by using a beta-adrenergic stimulant, e.g. isoproterenol As this involves an intravenous infusion there has been a search for simpler methods. After employing a variety of tests e.g. physical exercise and hyperventilation, Fitzgerald concluded that registration of heart rate after sublingual administration of nitroglycerine was best suited as it was least affected by atropinization [18] In the present study the nitroglycerine test has been employed in a different fashion from Fitzgerald's original description [17] as the patients assumed upright posture after the administration of nitroglycerine In the initial untreated state there was a good correlation between the response to nitroglycerine and the tachycardia produced by intravenous infusion of isoproterenol 3 μg/min. Both manoeuvres produced almost identical increase of heart rate in this situation. However during oral therapy with propranolol the increase of heart rate due to nitroglycerine was three times greater in spite of a significant correlation to the effect of isoproterenol. Naturally this reduces the usefulness of the nitroglycerine test as it indicates that the test may not be entirely specific. The greater increase of heart rate due to nitroglycerine may indicate that vagal withdrawal affected the response. Whether this difference is due to the fact that isoproterenol exerts its main effect on the resistance vessels whereas nitroglycerine is comparatively more effective on capacitance vessels [20] can not be answered by the present study From a practical point of view the discrepancy of heart rate response to isoproterenol and nitroglycerine during propranolol therapy indicates that the nitroglycerine test, as employed in the present study can only be used for crude estimations of the degree of beta-adrenergic blockade

The lack of correlation between the increase of heart rate during tilt and the response to isoproterenol and nitroglycerine indicated that tilt is not a useful manoeuvre for estimation of beta-adrenergic blockade.

Finally in view of recent reports regarding the lack of correlation between plasma propranolol and the degree of beta-adrenergic blockade ½[22] it is not surprising that the response to isoproterenol or nitroglycerine was not significantly correlated to plasma propranolol concentration.

Plasma propranolol concentration

Plasma propranolol concentration at the end of 4 weeks of oral treatment was significantly correlated to the reduction of systolic blood pressure both in the recumbent and standing position, while the overall reduction of diastolic blood pressure was not correlated to plasma propranolol concentration.

More interesting is the observation that once a certain reduction of blood pressure had occurred – as observed after 2 weeks of oral therapy in the present study – further reductions of both recumbent and standing systolic and diastolic blood pressure were directly linked to increasing plasma propranolol concentrations. This would indicate that increasing antihypertensive effect may be expected from increasing plasma propranolol concentrations. Obviously wide individual variations of absorption must be taken into consideration but this phenomenon could explain the impressive antihypertensive effect of propranolol reported in series where comparatively large doses were geven [61 81]

As mentioned previously the lack of correlation between plasma propranolol concentration and the degree of beta-adrenergic blockade, as estimated by the response to intravenous isoproterenol is in agreement with recent observations by others [83] This could indicate that tissue binding of propranolol is a stronger determinant of the degree of beta-adrenergic blockade than plasma concentrations. Another possibility is that metabolites with beta-blocking capacity e.g. 4-hydroxy propranolol contribute significantly to the degree of blockade thereby explaining the lack of correlation to plasma propranolol concentration.

CONCLUSIONS

Oral therapy with propranolol in essential hypertension caused significant reductions of systolic and diastolic blood pressure already during the first week of therapy The dosage used – 160 to 320 mg daily – caused a significant degree of beta-adrenergic blockade as demonstrated by the response to intravenous infusions of isoproterenol.

The reduction of systolic blood pressure was significantly correlated to plasma propranolol concentration. Hemodynamically acute administration of propranolol intravenously caused a significant reduction of cardiac output but no decrease of blood pressure due to an increase of systemic vascular resistance During prolonged oral therapy with propranolol cardiac output remained significantly reduced but peripheral resistance was re-adjusted towards the initial level resulting in a decrease of arterial pressure.

The mechanisms underlying this adjustment of systemic vascular resistance may be secondary to a resetting of baroreceptors

Reduction of plasma renin activity during prolonged oral treatment with propranolol is impressive. However a direct connection between the reduction of PRA and arterial pressure could not be demonstrated.

It was not possible to predict the antihypertensive response to propranolol in individual patients by studying parameters such as heart rate or cardiac output in the initial untreated state.

Finally heart rate response to sublingual nitroglycerine and assumption of upright posture was found to give only a crude estimation of the degree of beta-adrenergic blockade as tested by intravenous isoproterenol infusion.

GENERAL SUMMARY

This study has been concerned with the effects of acute and prolonged beta-adrenergic blockade with propranolol in essential hypertension. Fifteen male patients, all with mild to moderately severe essential hypertension, were included in the study. Initially placebo was given (single-blind) as the only treatment for 4 weeks. Hemodynamic parameters including cardiac output (dye dilution technique) and plasma renin activity were studied at the end of the placebo period. Propranolol was then given intravenously 0.22 mg/kg of body weight, and the studies of hemodynamics and renin were repeated. Following acute beta-adrenergic blockade there was a significant reduction of cardiac output with a compensatory increase of total peripheral resistance resulting in unchanged arterial pressure. The effect of tilt on plasma renin activity was diminished after acute beta-adrenergic blockade.

During oral treatment with propranolol significant reductions of systolic and diastolic blood pressure occurred already during the first week of therapy as judged from daily measurements of home blood pressure. The over all effect of 4 weeks of oral propranolol therapy on clinic blood pressure was a reduction of recumbent systolic pressure by 28.6 mm Hg ($p<0.0001$) and of diastolic pressure by 19.9 mm Hg ($p<0.0001$). Significant reductions of home blood pressure were seen as well.

Repeated hemodynamic investigations after 4 weeks of oral propranolol therapy revealed that cardiac output was still significantly reduced whereas systemic vascular resistance was re-adjusted to the initial level thereby resulting in a reduction of arterial pressure. Studies of the baroreceptor reflex suggested a resetting towards a greater sensitivity.

A marked decrease of plasma renin activity was seen after 4 weeks of oral propranolol therapy indicating that beta-adrenergic mechanisms play an important role in the mediation of renin release. The reduction of renin was not significantly correlated to the reduction of blood pressure indicating that the antihypertensive effect of propranolol in mild essential hypertension is not directly dependant on changes of plasma renin activity.

Attempts to define parameters of predictive value for the antihypertensive response to propranolol were not successful. No correlation was found between factors such as initial heart rate, cardiac output or the response to beta adrenergic stimulation and the reduction of blood pressure.

Studies of heart rate after sublingual administration of nitroglycerine and assumption of upright posture indicated that this will provide only a crude estimation of the degree of beta-adrenergic blockade.

Plasma propranolol concentration was found to be significantly correlated to the reduction of systolic blood pressure both in the recumbent and standing position whereas the change of diastolic blood pressure was not. On the whole, plasma propranolol concentration proved to be of little value as not even the degree of beta-adrenergic blockade, as assessed with intravenous isoproterenol infusion, was significantly correlated to it.

ACKNOWLEDGEMENTS

This work has been carried out in the stimulating milieu of the Department of Internal Medicine, Hypertension Section of the University of Michigan Medical Center in Ann Arbor Michigan.

Professor Andrew J Zweifler M.D and Associate Professor Stevo Julius, M.D have actively supported and supervised the work. They have also together with Professor Sibley W Hoobler M.D., provided the facilities for carrying out the work. I owe them a deep debt of gratitude.

Professor Lars Werkö M.D has given stimulating and positive critisism of the manuscripts as have Associate Professor Ulla Bengtsson, M.D and Professor Härje Bucht, M.D

Professor Bertil Hood, M.D introduced me to the field of hypertension and also provided the contact with the Hypertension Section in Ann Arbor

Professor Nils Johan Nilsson, M.D and Associate Professor Ramon Sivertsson, M.D introduced me to the field of clinical physiology and have also given valuable criticism of the work.

Professor James Conway M.D offered useful suggestions during the planning of the study

I would also like to thank Dr Stephen N Hunyor M.B., MRACP for stimulating support, Miss Nancy Basset, R.N and Mrs. Christina Willis, R.N for excellent patient care Mrs. Jane Bennet for efficient assistance in the laboratory Mr Ray Warshinsky for careful chemical analyses and Mr Charles N Ellis for skillful computer analysis of the data.

Furthermore, I wish to thank Mr Myrl Biggs of Ayerst Laboratories and Mr Per Erik Hörnkvist of ICI Pharmacenticals Division for valuable support and Mr Lars Erik Peterson for supervision of the statistical calculations

Finally financial support for this work was provided by Michigan Heart Association, Ayerst Laboratories, USPHS grant 5-M01 RR-42, USPHS grant 2-1 11-GM 15559 and the Swedish Medical Research Council grant K71 19R 3442.

REFERENCES

1. *Barret, A.M. & Calhon, J.A.* The biological properties of the optical isomers of propranolol and their effects on cardiac arrythmias. Brit. J. Pharmac. 34 43 1968.

2. *Birkenhäger W.H. Krauss X.H. Schalekamp M.A. D.H. Kolsters, G & Kroon, B U M* Antihypertensive effects of propranol. Observations on predictability Folia Med. Neerl. 14 67 1971

3. *Bourgoignie J.J.* Catanzano, F.J. & Perry H.M. Jr Renin-angiotensin-aldosterone system during chronic thiazide therapy of benign essential hypertension. Circulation 37 27 1968.

4. *Braun-Menendez, E., Fasciolo, J. C., Leloir L. F & Muñoz, J. M.* The substance causing renal hypertension. J. Physiol. 98 282, 1940

5. *Brunner H R., Laragh, J H Baer L., Newton, M A Goodwin, F T Krakoff L. R Bard, R H & Bühler F R.* Essential hypertension Renin and aldosterone heart attack and stroke. New Engl. J. Med 286 441 1972.

6. *Bühler F R., Laragh, J H Baer L., Vaughan, E D & Brunner H R.* Propranolol inhibition of renin secretion New Engl. J. Med 287 1209 1972

7. *Conway J* Effect of age on the response to propranolol Int J Clin Pharm 4 148 1970.

8. *Crane M G Harris, J J & Johns, V J J* Hyporeninemic hypertension Amer J Med 52 457 1972

9. *Cuthbert, M F & Peart, W S.* Studies on the identity of a vascular permeability factor of renal origin. Clin Sci 38 309 1970

10. *DeQuattro, V & Chan, S* Raised plasma-catecholamines in some patients with primary hypertension. Lancet I 806, 1972

11. *Djojosugito, A M F lkow B Oberg, B & White, S W* A comparison f blood viscosity measured in vitro and in a vascular bed Acta Physiol Scand. 78 70 1970

12. *Dorph, S & Binder C* E aluation of the hypotensive effect of beta-adrenergic blockade in hypertension Acta Med Scand 185-443 1969

13. *Eliash, S & Weinstock, M* Role of adrenergic neurone blockade in the hypotensive action of propranolol Brit. J Pharmac 43 287 1971

14. *Engelman, K Portnoy B & Sjoerdsma, A* Plasma catecholamine concentrations in patients with hypertension Circulation Res. 26-27 Suppl. I.141 1970

15. *Euler U S. von, Hellner S. & Purkhold, A* Excretion of noradrenaline in the urine in hypertension. Scand. J Clin. Lab Invest. 6.54 1954

16. *Fasciolo, J C., DeVito, E., Romero, J C & Cucchi, J.N* The renin content of the blood of humans and dogs under several conditions. Canad Med. Assoc. J 90.206 1964

17. *Fitzgerald, J D.*, A new test for the degree of adrenergic beta receptor blockade. Int. J Clin. Pharmacol. 4 125 1970.

18. *Fitzgerald, J D Rowlands, D J Hewitt, G & Davies, E T L.* Heart rate response to certain physiological manoeuvres Proposed measure of degree of beta-receptor blockade. Brit. Heart J 33.614 1971

19. *Fries, E D* Hemodynamics in hypertension. Phys. Rev 40.27 1960.

20. *Frohlich, E D Tarazi, R. C., Dustan, H P & Page I H.* The paradox of beta-adrenergic blockade in hypertension. Circulation 37 417 1968.

21. *Gavras, H Brown, J J Lever A F Macadam, R. F & Robertson, J I S* Acute renal failure, tubular necrosis, and myocardial infarction induced in the rabbit by intravenous angiotensin II Lancet II 19 1971.

22. *Giese, J* Acute hypertensive vascular disease I Relation between blood pressure changes and vascular lesions in different forms of acute hypertension. Acta Path. Microbiol. Scand. 62.481 1964

23. *Goldblatt, H Lynch, J Hanzal, R. F & Summerville, W W* Studies on experimental hypertension. I. Production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exper Med 59 347 1934

24. *Haber E., Koerner T Page L B Kliman, B & Purnode, A* Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J Clin. Endocrin. Metabol. 29 1349 1669

25. *Hansson, L., Malmcrona, R Olander R R Werkö, L Westerlund, A Åberg, H & Hood B* Propranolol in hypertension. Report on 158 patients treated up to one year Klin. Wschr 50.364 1972.

26. *Helmer O M.* Renin activity in blood from patients with hypertension. Canad. Med. Assoc. J 90:221 1964.

27 *Humphreys, G S. & Delvin, D. G* Ineffectiveness of propranolol in hypertensive Jamaicans. Brit. Med. J 1.601 1968.

28 *Johansson, G. & Öberg, B* Comparative effects of isoprenaline and nitroglycerine on consecutive vascular sections in the skeletal muscle of the cat. Angiologica 5 161 1968.

29 *Jose, A Crout, J R. & Kaplan, N M* Suppressed plasma renin activity in essential hypertension. Roles of plasma volume blood pressure and sympathetic nervous system. Ann. Int. Med. 72.9 1970.

30. *Julius, S. & Conway J* Hemodynamic studies in patients with borderline blood pressure elevation. Circulation 38.282, 1968.

31 *Kaneko, Y Ikeda, T Takeda, T & Ueda, H* Renin release during acute reduction of arterial pressure in normotensive subjects and patients with renovascular hypertension. J Clin. Invest. 46·705 1967

32. *Küchel, O., Fishman, L. M Liddle, G W & Michelakis, A* Effect of diazoxide on plasma renin activity in hypertensive patients. Ann. Int. Med. 67 791 1967

33 *Laragh, J H., Angers, M Kelley W G & Lieberman, S* Hypotensive agents and pressor substances. The effect of epinephrine norepinephrine, angiotensin II and others on the secretory rat of aldosterone in man. J A. M. A. 174.234 1960.

34. *Laragh, J H* The role of aldosterone in man: Evidence for regulation of electrolyte balance and arterial pressure by renal-adrenal system which may be involved in malignant hypertension. J A. M A. 174 293 1960.

35 *Laragh, J H., Ulrich, S Jamiszewicz, V Deming, Q. B Kelley W G & Lieberman, S.* Aldosterone secretion in primary and malignant hypertension. J Clin. Invest 39 1091 1960

36. *Laragh, J H., Sealey J E & Sommers, S. C.* Patterns of adrenal secretion and urinary excretion of aldosterone and plasma renin activity in normal and hypertensive subjects Circulation Res. 18 Suppl I 158 1966

37 *Lund-Johansen, P* Hemodynamics in early essential hypertension Acta Med. Scand Suppl 482 8 1967

38 *Maxwell, J A Hunter, A & Hollander W E* Post hydralazine renin release in the diagnosis of renovascular hypertension. Am Surg 70·409 1969

39 *Maxon, G M C Mikawa, A & Yasuda, H.* Experimental vascular disease elicited by aldosterone and renin. Endocrinology 71 505 1962

40 *Michelakis, A M & McAllister R G* The effect of chronic adrenergic receptor blockade on plasma renin activity in man J Clin Endocrin. Metabol 34 386 1972

41 *Mohammed, S., Fasola, A F Privitera, P J Lipicky R. J M rtz, B L. Gaffney T E.* Effect of methyldopa on plasma renin activity in man. Circulation Res. 25:543 1969

42. *Moore, J W Kinsman, J M Hamilton, W F & Spurling, R G* Studies on the circulation. II Cardiac output determinations. Amer J Physiol. 89.331 1929

43 *Mulrow P J Ganong, W F Cera, G & Kuljian A.* The nature of the aldosterone-stimulating factor in dog kidneys. J Clin. Invest. 41:505 1962.

44 *Onesti, G Schwartz, A B Kim, K. E., Paz-Martinez, V & Swartz, C.* Antihypertensive effect of clonidine. Circulation Res. 28–29 Suppl. II. 53 1971

45 *Page I. H & Helmer O M* Crystalline pressor substance (angiotonin) resulting from reaction between renin and renin-activator J Exper Med. 71.29 1940.

46. *Paget, G E.* Carcinogenic action of pronethalol. Brit. Med. J I.1266 1963

47 *Pickering G W* High Blood Pressure. Churchill, London. 1968. pp 717

48. *Prichard, B N. C.* Hypotensive action of pronethalol. Brit. Med. J I.1227 1964

49 *Prichard, B. N C. & Gillam, P M. S.* The use of propranolol in the treatment of hypertension. Brit. Med. J II. 725 1964.

50. *Prichard, B N C. & Gillam, P M. S.* Propranolol in hypertension. Amer J Cardiol. 18.387 1966.

51 *Prichard, B. N. C. & Gillam, P M S.* Treatment of hypertension with propranolol. Brit. Med. J I.7 1969

52. *Prichard, B N. C., Boakes, A J & Day G* Practolol in the therapy of hypertension. Postgrad. J Med. 47 Suppl. 84 1971

53 *Sannerstedt, R* Hemodynamic response to exercise in patients with arterial hypertension. Acta Med. Scand. Suppl. 458 1 1966.

54. *Sannerstedt, R Julius, S. & Conway, J* Hemodynamic responses to tilt and beta-adrenergic blockade in young patients with borderline hypertension. Circulation 42 1057 1970.

55 *Schröder G & Werkö, L.* Nethalide a beta adrenergic blocking agent. Clin. Pharmacol. Therap. 5 159 1964.

56 *Séderberg-Olsen, P & Ibsen, H* Plasma volume and extracellular fluid volume during long-term treatment with propranolol in essential hypertension. Cli. Sci. 43 165 1972.

57 *Shand, D G Nicholls, E M & Oates, J A* Plasma propranolol levels in adults With observations in four children Clin Pharmac Ther 11 112, 1970

58. *Simpson, T A Tait, J F Wettstein, A Neher R Euw J von, & Reichstein, T* Isolierung eines neuen kristallisierten Hormons aus Nebennieren mit be-

sonderes hoher Wirksamkeit auf den Mineralstoffwechsel. Experentia 9:333 1953.

59 Snertsson, R. The hemodynamic importance of structural vascular changes. Acta Physiol. Scand. Suppl. 343 1 1970.

60 Skeggs, L. T Jr Kahn, J R & Shumway N P The isolation and assay of hypertensin from blood. J Exper Med. 95.241 1952.

61 Skeggs, L. T Jr Marsh, W H Kahn, J R & Shumway N P The existence of two forms of hypertensin. J Exper. Med. 99.275 1954

62. Skeggs, L. T Jr Kahn, J R & Shumway N P Preparation and function of the hypertensin-converting enzyme. J Exper Med. 103.295 1956.

63 Sleight, P Gribbin, B & Pickering, T G Baroreflex sensitivity in normal and hypertensive man the effect of beta-adrenergic blockade on reflex sensitivity Postgrad. Med J 47 Suppl. 79 1971

64. Smyth, H S. Sleight, P & Pickering, G W Reflex regulation of arterial pressure during sleep in man Circulation Res. 24 109 1969

65 Stockigt, J R. Collins, R. D & Biglieri, E. G Determination of plasma renin concentration by angiotensin I immunoassay Circulation Res. 28-29 suppl. II.175 1971

66. Tarazi, R. C. Dustan, H P & Frohlich, E. D Long term thiazide therapy in essential hypertension. Evidence for persistent alteration in plasma volume and renin activity Circulation 41 709 1970.

67 Tarazi, R. C. Frohlich, E. D & Dustan, H. P Plasma volume changes and beta-adrenergic blockade. Amer Heart J 82.770, 1971

68. Tarazi, R. C. & Dustan, H. P Beta-adrenergic blockade in hypertension. Practical and theoretical implications of long-term hemodynamic variation. Amer J Cardiol. 29 633 1972.

69 Tarazi, R. C. Dustan, H P Frohlich, E. D & Bravo, E. L. Plasma renin and arterial pressure response to propranolol. Circulation 45-47 Suppl. II.70 1972.

70. Tibblin, G Bergentz, S.-E. Bjure, J & Wilhelmsen, L. Hematocrit, plasma protein, plasma volume and viscosity in early hypertensive disease. Amer Heart J 72:165 1966.

71 Tibblin, G & Åblad, B Antihypertensive therapy with alprenolol a beta-adrenergic antagonist. Acta Med. Scand. 186.451 1969

72. Tigerstedt, R & Bergman P Niere und Kreislauf. Skandinav Arch. Physiol. 8.223 1898.

73 Tobian, L. Jr & Binion, J T Tissue cations and water in arterial hypertension. Circulation 5 754 1952.

74. Tobian, L. Relationship of the juxtaglomerular apparatus to renin and angiotensin. Circulation 25 189 1962.

75 Ulrych, M Frohlich, E. D Dustan, H P & Page, I.H Immediate hemodynamic effects of beta-adrenergic blockade with propranolol in normotensive and hypertensive man. Circulation 37·411 1968.

76. Veyrat, R DeChamplain, J Boucher R & Genest J Measurement of human arterial renin activity in some physiological and pathological states. Canad. Med. Assoc. J 90.215 1964

77 Waal-Manning, H J Lack of effect of d-propranolol on blood pressure and pulse rate in hypertensive patients. Proc. Univ Otago Med. School, 48:80 1970.

78. Wallin, B G Delius, W & Hagbarth, K.-E. Comparison of sympathetic nerve activity in normo- and hypertensive subjects. (Submitted for publication)

79 Weinberger M & Perkins, B. The correlation of plasma renin activity with hypertensive retinopathy Clin. Res. 20:727 1972.

80. Winer N Chokshi, D S. Yoon, M S. & Freedman, A D Adrenergic receptor mediation of renin release. J Endocrin. Metabol. 29·1168 1969

81 Zacharias, F J Cowen, K J Prestt, J Vickers, J & Wall, B G Propranolol in hypertension. A study of long-term therapy 1964-1970 Amer Heart J 83 755 1972.

82. Zacharias, R & Koch-Weser J Relation of propranolol plasma level to Blockade during oral therapy Pharmacology 7 178, 1972.